

From the Laboratory for Experimental Brain Research, E-blocket, and
the Department of Neurosurgery, University of Lund, Lund, Sweden

Local Cerebral Blood Flow in the Recirculation Period Following Complete and Incomplete Cerebral Ischemia

An Experimental Study in Rats

ERIK KÅGSTRÖM

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C O N T E N T S

Introduction	5
Objectives	11
Methods	12
Experimental Groups	14
Results and Discussion	15
Conclusions	24
Acknowledgements	25
References	26
Paper I	33
Paper II	65
Paper III	91
Paper IV	119

The present thesis is based on the following papers:

- I. Kågström E, Smith ML, & Siesjö BK (1982) Local cerebral blood flow in the recovery period following complete ischemia in the rat. J Cereb Blood Flow Metabol, submitted for publication.
- II. Kågström E, Smith ML, & Siesjö BK (1982) Recirculation in the brain following incomplete ischemia in the rat. J Cereb Blood Flow Metab, submitted for publication.
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- IV. Kågström E, Smith ML, & Siesjö BK (1982) Cerebral circulatory responses to hypercapnia and hypoxia in the recovery period following complete and incomplete ischemia in the rat.
Manuskript.

INTRODUCTION

Twenty years ago it was generally accepted that the brain will not tolerate more than 5-7 minutes of complete ischemia or anoxia. Research results during the last decades have necessitated revision of this concept. Considerable restitution of neurological function was reported following 20-25 min of complete ischemia in dogs and monkeys (Neely and Youmans 1963, Miller and Myers 1970). Under "optimal" experimental conditions 60 min of complete ischemia was found to be compatible with recovery of spontaneous EEG and somatosensory evoked potentials from the cortex (Hossmann et al 1973). However, even if gross energy metabolism of the brain is restored and single brain neurons may appear to function normally, integrated brain function will not return after such long periods of ischemia. It was suggested (Hossmann and Olsson 1970, Olsson and Hossmann 1971) that optimal conditions for recovery from cerebral ischemia requires that ischemia commences abruptly and is complete, that blood is not allowed to stagnate in the cerebral vessels, and that the perfusion pressure is quickly restored to (or above) normal.

These and other observations have favoured the view that the permanent damage incurred by ischemia to a considerable extent is determined by events, both circulatory and metabolic, that occur during the postischemic period. This has created a more optimistic outlook on the possibilities to ameliorate the often disastrous consequences of stroke. It is not surprising, therefore, that attempts are currently made to unravel the mechanisms, during ischemia and during recirculation, which are of significance for the final outcome of a cerebrovascular insult. A significant proportion of this research is centered on purely circulatory events, and on the question whether or not an inadequate tissue perfusion in the postischemic period is responsible for part of the final cell damage.

Models for induction of experimental ischemia

Among existing experimental models for induction of cerebral ischemia, occlusion of a single artery (usually the middle cerebral artery, MCA) is the one which most closely resembles clinical stroke. A vast literature exists which is based on observations from experiments of this type (for ref. see Hayakama and Waltz 1975). Disadvantages with this method are the great variability in the extension of

lesion incurred and the fact that intracranial intervention is required. It lends itself primarily to experiments on big animals, e.g. monkeys and cats, but the development of microsurgical techniques has made it possible to use small animals as well (Tamura et al. 1981).

A unique type of ischemia can be induced in the mongolian gerbil which has an incomplete circle of Willis. Unilateral carotid occlusion will provoke signs of stroke in about 40% of these animals (Levine and Payan 1966, Kahn 1972, Levy et al. 1979).

Experimental models with more global types of ischemia imitate clinical conditions with increased intracranial pressure, extreme arterial hypotension, cardiac failure or transient total circulatory arrest. In many respects these models have advantages over the regional models. Reproducibility is easier to achieve. Selective vulnerability in different brain structures can be explored. Small animals can be used. The severity of ischemia can be varied from complete to moderate.

Among methods for induction of complete ischemia allowing recovery, the CSF compression method is the simplest. It only requires infusion of artificial CSF into the subarachnoid space at a rate that raises the intracranial pressure to a level above the systolic blood pressure (Neely and Youmans 1963, Kramer and Tynman 1967, Ljunggren et al. 1974a, Marshall et al. 1975, Hallenbeck and Bradley 1977). Ventricular fibrillation may be convenient for short ischemic episodes but problems with resuscitation will arise if extended periods are practised (Wolfe 1960, Safar et al. 1976). More often total circulatory arrest is accomplished by occlusion of the aorta and the venae cavae (Brockman and Jude 1960, Snyder et al. 1975, Wade et al. 1975). Due to the abundance of collaterals to the cerebral arteries from the neck and head and within the spinal canal, isolation of the intracranial circulation from the rest of the body usually requires complicated surgical interventions (Wright 1965, Hossmann and Olsson 1970). A satisfactory interruption of collateral flow to the brain can be achieved by arterial occlusion combined with moderate arterial hypotension (Hossmann et al. 1976). Alternatively, clamping of the arteries to the head may be supplemented by a pressure cuff around the neck (Kowada et al. 1968).

Generalized incomplete ischemia is technically easier to achieve than complete ischemia, but shares with regional ischemia the

difficulties in obtaining reproducibility. A combination of bilateral carotid occlusion and induced hypotension in rats was used by Eklöf and Siesjö (1972) and by Nordström and Siesjö (1978). Pulsinelli et al. (1982a) induced severe incomplete forebrain ischemia in unanesthetized animals by bilateral carotid artery clamping after previous permanent occlusion of the vertebral arteries in rats.

Metabolic events in the brain during and following ischemia

The maintenance of a normal cerebral metabolism requires an uninterrupted supply of oxygen and substrate by the circulation. Cessation of the mitochondrial energy production, required for this metabolism, is an early event during ischemia. I will make a brief comment on metabolic changes occurring during and in the recirculation period following ischemia.

After the onset of complete ischemia the cellular energy sources are quickly exhausted. Most of the energy-rich phosphates, such as phosphocreatin and ATP, have disappeared within two minutes (Lowry et al. 1964, Siesjö 1978). When mitochondrial respiration fails, anaerobic glycolysis is stimulated and affords some continued ATP production. This results in accumulation of lactic acid in the brain tissue. During complete ischemia the lactate concentration reaches levels of about 12-13 $\mu\text{mol.g}^{-1}$ after a couple of minutes. Since the tissue stores of glucose and glycogen are then depleted no further increase occurs even if ischemia is prolonged (Ljunggren et al. 1974a). If the ischemia is incomplete there is some continued supply of substrate (glucose) and the lactate concentration may reach high levels ($>30\mu\text{mol.g}^{-1}$) (Nordström and Siesjö 1978). Comparable lactate concentrations may be encountered in severe hypoxia even if cerebral blood flow does not fall below control values (Salford and Siesjö 1974). The amount of lactate accumulated in severe ischemia depends on blood glucose concentrations, residual blood flow, and the duration of ischemia (see Rehncróna et al. 1981).

If brain perfusion is restored mitochondrial function may return with subsequent recovery of labile phosphates. Under optimal experimental conditions considerable metabolic recovery may occur even after ischemic periods of 30-60 min (Hinzen et al. 1972, Hossmann and Kleihues 1973). In rats, a surprisingly complete restoration of cerebral energy state is observed after ischemic periods of 15 min (Ljunggren et al. 1974b) and even 30 min (Nordström et al 1978a).

However, after extended ischemia the adenine nucleotide pool (and, thereby, the ATP concentration) may remain depressed for long periods (Kleihues et al. 1974). Furthermore, although recovery of energy state is accompanied by reduction of tissue lactate, some lactacidosis may persist for hours if the preceding ischemia is of long duration (Hossmann and Kleihues 1973, Kleihues et al. 1974).

As mentioned above, revival of mitochondrial respiration is not necessarily associated with recovery of brain function. Integrated neuronal function is much less tolerant to the ischemic injury than the energy production mechanisms.

Recovery from ischemia, be it metabolic, electrophysiological or functional, is influenced by several factors. Most important, of course, is the duration of ischemia. Hypothermia has clearly a protective action (e.g. Hirsch et al. 1957, Boyd and Connolly 1962). It is currently debated whether barbiturates increase the resistance of the brain to ischemia, and whether the protective effect of anaesthetics, if any, is due to a reduction of cerebral metabolism only or if other mechanisms are responsible as well (see Nordström and Siesjö 1978, Steen et al. 1978). The best interpretation of available data is that some anaesthetics, particularly the barbiturates, offer some protection in situations of regional and incomplete ischemia, but that they have no effect in situations of global, complete ischemia.

The apparently paradoxical observation that severe incomplete ischemia could cause more severe tissue damage than complete ischemia (Hossmann and Kleihues 1973, Nordström et al. 1978b, Rehncrona et al. 1979) has been explained by the difference in tissue lactate accumulation (Rehncrona et al. 1981). This can also explain the beneficial effect of food withdrawal before induction of ischemia on the functional damage incurred (Myers and Yamaguchi 1976), and the detrimental effect of glucose administration (Siemkovicz and Hansen 1978, Myers 1979, Rehncrona et al. 1981, Kalimo et al. 1981). It should be recalled that glycolysis in the brain is retarded during hypothermia and in barbiturate intoxication. This may contribute to the protection afforded in these conditions.

It is currently debated whether the consequences of a deranged phospholipid metabolism influence the outcome of ischemia. The proposal was made that the incomplete ischemia associated with MCA occlusion leads to formation of free radicals with resulting cell

damage due to lipid peroxidation (Demopoulos et al. 1977, Flamm et al. 1978) or, alternatively that such reactions may be triggered during the recirculation/reoxidation period following transient ischemia (Nordström et al 1978b) Although subsequent experiments have not provided support for this proposal (Rehncrona et al. 1980, Cooper et al. 1980), continued interest is focussed on phospholipid metabolism during ischemia.

Ischemia of a severity that disrupts energy balance at the cellular level leads to accumulation of free fatty acids (FFA) in the brain and of particular interest is the increase of the concentration of arachidonic acid, the substrate for prostaglandin synthesis (Bazan 1970, Marion and Wolfe 1979). Massive accumulation of prostaglandins occur in the brain during the postischemic period (Gaudet et al. 1980). It is clear, therefore, that conditions are at hand for a potentially injurious sequence of postischemic reactions that may involve an imbalance between thromboxane A₂ (vasoconstrictory and proaggregatory) and prostacyclin (vasodilatory and antiaggregatory), as well as for initiating free radical reactions, which may provoke damage to vascular and parenchymal cells (see Siesjö 1981).

Circulatory events during reperfusion

Reinstitution of an adequate cerebral perfusion pressure is a sine qua non for recovery from ischemia. Clinical and experimental studies have revealed a number of circulatory disturbances that may occur during the recirculation period. May be the most well known is the initial "reactive hyperemia" (Hossmann et al. 1973, Hossmann and Zimmermann 1974, Cuypers and Matakas 1974). A similar hyperemic response was described to occur also following transient increase of the intracranial pressure (Häggendal et al. 1970, Zwetnow 1970) and the "luxury-perfusion syndrome" described by Lassen (1966) is possibly of related nature. Evidence exists that the initial hyperemia is a prerequisite for postischemic recovery, at least after long ischemic periods (Hossmann et al. 1973).

A second type of postischemic circulatory disorder was described by Ames et al. (1968). These authors found areas of deficient perfusion when rabbit brains were perfused with colloidal carbon at the end of ischemia. This "no-reflow phenomenon" mainly involved the basal ganglia but could occupy cortical structures as well. In a

series of publications from the same group it was argued that the perfusion defects were caused by erythrocyte stasis and increased blood viscosity in vessels whose lumina were narrowed due to swelling of perivascular glia cells. They also suggested that the extension of the perfusion defects will be reduced if reperfusion occurs at normal perfusion pressures (Fischer et al. 1979). Hemodilution prior to ischemia also seemed to increase the amount of perfused tissue.

During the last ten years studies on postischemic cerebral circulation have focussed interest on another type of perfusion disorder. Thus, the initial reactive hyperemia was found to be succeeded by a secondary decrease in CBF, called "delayed hypoperfusion" (Zimmer et al. 1971, Hossmann et al. 1973, Snyder et al. 1975, Nordström and Rehncrona 1977). During this period overall CBF is reduced to about 50% of preischemic control values. This secondary hypoperfusion may persist for 4-8 hours (Levy et al. 1979, Pulsinelli et al. 1982a). It has been postulated that the CO₂ reactivity of the cerebral vessel is abolished in this period (Hossmann et al. 1973, Nemoto et al. 1975), at least if the preceding period of ischemia exceeds 5 min (see Miller et al. 1980a). The vascular unreactivity also includes failing response to various drugs with known vasodilator effect (Hossmann et al. 1973, Takagi et al. 1977). Most observations concerning CO₂ reactivity following ischemia are based on models with complete ischemia with measurements of global CBF only. The cause of the delayed hypoperfusion remains unknown.

It has not been clarified what pathogenic significance these postischemic circulatory disturbances may have. However, the existence of no-reflow areas will obviously prolong the ischemic period in affected regions and, consequently, reduce the chances of recovery. It has been speculated, also, that the delayed hypoperfusion may add a secondary ischemic insult to the brain tissue, particularly if the reduction in CBF is not matched by a corresponding lowering of the metabolic rate (Snyder et al. 1975, Hossmann et al. 1976, Nordström and Rehncrona 1977, Pulsinelli et al. 1982 a and b).

The postischemic perfusion disorders are of special importance in view of the fact that the final cell damage following ischemia seems to "mature" over hours and days (Ito et al. 1975, Pulsinelli et al. 1982 b). In this context, it is also of great interest that experimental studies of this type have re-emphasized the fact that

certain regions in the brain, e.g. the neocortex, hippocampus, and caudoputamen, show selective vulnerability to ischemic insults (Brierley et al. 1975, Pulsinelli et al. 1982b)

OBJECTIVES

The objectives of the present studies were to explore the postischemic circulatory disturbances described above with an autoradiographic technique which allows estimation of CBF changes on the local level. In particular we wanted to provide answers to the following questions:

1. Can a true no-reflow phenomenon be observed with the technique for measurement of local CBF used in these studies? If this is so, is the phenomenon peculiar to complete ischemia, or does it follow upon incomplete ischemia as well?
2. Are there regional differences in CBF during the period of delayed hypoperfusion, even if the preceding ischemia affects all structures equally?
3. Is the nutritional state of the animals before induction of ischemia of significance for the severity of postischemic circulatory disturbances?
4. Is the postulated unreactivity of cerebral vessels to CO_2 and other agents during delayed hypoperfusion a general phenomenon, or are there differences at the local level?
5. Can inhibition of prostaglandin synthesis ameliorate postischemic perfusion disorders?
6. Is there any relation between the documented selective vulnerability of brain structures and the distribution of postischemic regional circulatory disorders?

To meet these objectives, and to be able to provide answers to these questions, we have performed a series of experiments using the models for complete and incomplete ischemia in rats developed in our laboratory. A CSF compression model was described by Ljunggren et al. (1974a) and a model for incomplete ischemia by carotid clamping combined with hypotension was presented by Nordström and Siesjö (1978). We also designed and used a new model in which complete ischemia was induced in rats by four-vessel-occlusion plus hypotension. A description of the experimental groups will be given below.

METHODS

Animals, operative and analytical techniques

The experiments were performed on male Wistar rats. "Fed" rats had free access to water and rat pellets until operation, "fasted" rats had their food supply withdrawn during the night before the operation. Anesthesia was induced with 3% halothane. After tracheostomy the animals were artificially ventilated with a mixture of 0.7% halothane in 30% O₂ and 70% N₂O during the subsequent operation. The femoral arteries, femoral veins, and one brachial artery were cannulated for blood sampling, continuous blood pressure recording, infusions, and injections. Copper screws were inserted in the skull bone for EEG recordings. The animals were paralyzed with tubocurarine chloride and heparinized. Following operation the halothane supply was discontinued and the animals were kept in a steady state (PaO₂ about 100 mm Hg, PCO₂ between 33 and 40 mm Hg) for 30 min before induction of ischemia. The body temperature was kept close to 37°C and a heating bulb was placed over the head of the animal to prevent the intracranial temperature from falling during the ischemic period. Excessive systemic acidosis during recirculation was counteracted by repeated injections of small doses of sodium bicarbonate and, if necessary, ventilation volume was increased to compensate for an increase in PCO₂.

Arterial blood was sampled anaerobically for blood gas and pH determinations. Analysis of blood concentrations of glucose, lactate, and pyruvate was performed after sampling of arterial blood directly into liquid nitrogen.

In experiments designed for studies of cerebral metabolites the brains were frozen in situ according to Pontén et al (1973) and then chiselled out and stored at -80°C until extraction and analyses with the specific, enzymatic, fluorometric techniques of Lowry and Passonneau (1972).

Statistical comparison was performed using Student's t-test.

Reversible complete CSF compression ischemia

Compression ischemia was induced with the method described by Ljunggren et al. (1974a). Prewarmed artificial CSF was infused into the cisterna magna through a double-barrelled needle which allowed

continuous recording of the CSF pressure. The vasopressor response to increased intracranial pressure was counteracted by i.v. infusion of ArfonadR, if necessary complemented by arterial bleeding. To restore the cerebral perfusion pressure we interrupted the cisternal infusion and normalized the blood pressure.

Reversible complete four-vessel-occlusion ischemia

The upper part of the sternum was removed to allow approach to the anterior aspect of the caudal part of the cervical column. The transverse processes of the 6th vertebra and the vertebral arteries and veins were indentified on both sides. A thread was applied behind the oesophagus but in front of the common carotid arteries (leaving the nerve trunks outside) and, with a needle, it was then drawn through the paravertebral muscles bilaterally at the level C6-C7. Four-vessel-occlusion was accomplished by tying the thread in the back of the animal. To compress collaterals a cotton band was tied hard around the neck above the ligature. The trachea was kept outside the cotton band. Before vessel occlusion the blood pressure was lowered to about 50 mm Hg by i.v. infusion of Arfonad and kept at that level during the ischemia. Arfonad infusion was often supplemented by arterial bleeding. We restored the circulation in the brain by releasing the ties and normalizing the blood pressure with blood infusion.

Reversible incomplete ischemia

Induction of incomplete ischemia was performed by clamping both common carotid arteries (Nordström and Siesjö 1978). Simultaneously the blood pressure was decreased to 50 mm Hg by arterial bleeding and kept at that level during the ischemic period using a servo-system for automatic extraction and reinfusion of blood. To restore brain perfusion we removed the clamps and normalized the blood pressure by reinfusion of blood.

Measurement of local CBF

We measured local CBF with the technique described by Landau et al. (1955) and Sakurada et al. (1978). Modifications of procedures applied in these studies have been described in previous publications from the laboratory (Abdul-Rahman et al. 1979, Ingvar et al 1981). 30-50 μ Ci of ^{14}C -iodoantipyrin, dispensed in 0.5-1.0 ml of

Krebs-Hensleit solution was infused at a constant rate for 20-60 sec. If high flow rates were expected a short infusion period was chosen, and vice versa. Blood samples were obtained from the brachial artery before and at frequent intervals during the isotope infusion. At the end of the infusion period the animal was decapitated, whereafter the brain was removed from the skull and immediately immersed in isopentane chilled to -55 to -65°C. The ^{14}C activity in brain tissue was evaluated by autoradiography. Twenty μm thick slices of brain were cut in a sledge microtome at -200°C and applied to an X ray film which was then exposed for one week. A series of calibrated ^{14}C -standards was applied to each film together with the brain slices. After exposure the film was evaluated manually with a densitometer (McBeth, 1 mm aperture). Calculation of local CBF in 24 different brain structures was then performed as described by Sakurada et al. (1978).

Experimental groups

With few exceptions each experimental group in these studies comprised 6 animals (4 animals in some experiments designed for studies of cerebral metabolites). Our objectives made it important to choose ischemic insults that allowed rapid resumption of normal perfusion pressures and we wished to avoid the pitfalls of a slowly deteriorating circulation as this may occur following long ischemic periods. Therefore, we chose a 15 min period of ischemia as a standard procedure in all models. This insult will allow restitution of cerebral energy state and should be compatible with considerable functional recovery, at least under optimal experimental conditions. Shorter and longer periods were also applied in experiments with the CSF compression model (paper I) for special purposes (see below).

We measured local CBF during incomplete ischemia (to characterize the model) and in the recirculation period following ischemia of the 3 types used. We chose recovery periods of 5 min to characterize flow patterns in the immediate recirculation phase, and 60 min for analysis of the delayed hypoperfusion. Following complete CSF compression ischemia an additional group was studied after 90 min of recovery.

In the first two papers we studied the "natural course" of local CBF changes following complete (paper I) and incomplete (paper II) ischemia. In the latter, fed as well as fasted animals were used,

to elucidate the influence of different levels of lactate accumulation in the brain tissue on postischemic flow rates.

Paper III deals with the effect of inhibition of prostaglandin synthesis on postischemic perfusion impairments. The animals were injected with indomethacin before induction of ischemia and lCBF was measured in the immediate recovery period following complete compression ischemia and in immediate as well as late recovery periods following incomplete ischemia.

In paper IV, finally, we examined the reactivity of the cerebral vessels during the period of delayed hypoperfusion and exposed the animals to short episodes (2-3 min) of hypercapnia or hypoxia before measurement of lCBF.

Analysis of cortical tissue metabolites were performed with two objectives. First, to assess the efficacy of four-vessel-occlusion ischemia, cortical tissue concentrations of labile phosphates as well as of glucose, pyruvate, and lactate were measured at the end of 15 min ischemia and compared with results obtained from shamoperated animals. Second, to estimate the accuracy of our results from measurements of local CBF during incomplete ischemia, cortical metabolites were analysed in animals exposed to 15 min of incomplete ischemia and the results compared with those obtained in control animals.

R E S U L T S a n d D I S C U S S I O N

In this chapter I will summarize the results obtained in the four papers and discuss their relevance for the questions posed in the introduction. Before doing so it seems justified to make some comments on the models for induction of ischemia used in these studies.

CSF compression ischemia implies, not only total arrest of the intracranial circulation, but also an abrupt rise in the intracranial pressure and, probably, an acute dislocation of brain structures at the beginning of CSF infusion. Furthermore, some of the artificial CSF infused is absorbed into the general circulation with an ensuing hemodilution. Thus, the erythrocyte volume fraction drops from about 50% before to about 30% at the end of the ischemia and during recirculation it gradually increases but, after 60 min of recovery, it is usually still below 40%. The effect of this hemodi-

lution (which tends to increase flow rates) should be considered when comparison is made between flow values obtained in the recirculation period following this and the other types of ischemia.

The four-vessel-occlusion model, described in paper I, was designed with the particular purpose to confirm that the CBF impairments observed following CSF compression ischemia were due to the ischemia per se and not consequences of other factors inherent in the method. It was mentioned in the introduction that the abundance of collaterals to the cerebral vasculature makes it difficult to induce complete ischemia with vascular occlusion. We have checked the efficacy of our four-vessel-occlusion model in two ways. The presence of any residual blood flow in the brain during ischemia was evaluated in two animals with the autoradiographic technique described above. The films showed no activity in the brains. We also examined the accumulation of lactate in the brain tissue at the end of 15 min of four-vessel-occlusion. The results showed that the lactate concentrations in the brains did not increase more (by about $13 \mu\text{mol.g}^{-1}$), compared with shamoperated animals, than could be expected if ischemia is complete. We conclude from these results that four-vessel-occlusion with the model used induces complete ischemia.

The model for incomplete forebrain ischemia used maintains some circulation to brain stem structures and cerebellum, but gives only a trickle of flow to cortical structures (Nordström and Rehncrona 1977)). The method is presently being adopted for long-term survival studies and therefore we deemed it desirable to further characterize this model in terms of physiological and biochemical variables, and to obtain more detailed information on local flow rates during as well as following ischemia. The results of this study (paper II) showed that during incomplete ischemia lCBF is reduced to values below 5% of control in all cerebral cortical structures, as well as in caudoputamen, amygdala, and hippocampus. Many structures had such low calculated flow rates that it becomes a matter of speculation whether the ischemia was complete or incomplete. Values below 15% of control were obtained in parts of the thalamus and in structures in the rostral parts of the brain stem. Higher values (>15% of control) were seen in the hypothalamus, the cerebellum, and in caudal brain stem nuclei (for details, see paper II, fig.4). Interanimal variations were small in cerebral cortical structures but larger in caudal structures,

reflecting variable continued supply from the vertebral system and from collaterals.

Although the autoradiographic technique used in these studies is well suited to elucidate the problems studied in the present context, it does not accurately resolve differences in CBF when flow rates fall below 10% of control. It is of importance, therefore, that deductions regarding CBF during severe incomplete ischemia can be made from results on the amount of lactate accumulated in the ischemic brain tissue. From calculations of the amounts of glucose that must have been translocated from blood to brain tissue during ischemia to account for the tissue lactate changes, we could deduce that the low CBF values recorded in many structures represent reasonable estimations of true average perfusion rates.

Another methodological aspect is worth consideration. It was shown by Dahlgren et al. (1981a) that nitrous oxide anaesthesia provokes increased flow rates in several cortical structures. These were the same structures in which we found (paper I) that a very short-lasting (2-3 sec) increase of the intracranial pressure above mean arterial pressure was followed by a reduction in CBF measured 5 min later. These observations suggest that for these structures the true decrease in CBF in the 60 min recovery period (and the increase in the initial phase) should be assessed by comparison with CBF values obtained in awake, unaenesthetized animals.

In all the ischemic models used, an adequate perfusion pressure was resumed within 2 min following the end of ischemia.

With this information at hand we can proceed to discuss the results. Observations during the immediate (5 min recovery) and late (60 min recovery) postischemic periods will be discussed in turn and under separate headings.

I. FIVE MIN RECOVERY - hyperemia versus no-reflow

The autographic films from animals subjected to 15 min of complete compression ischemia, and allowed 5 min of recirculation, revealed the presence of perfusion defects, which could be verified by densitometric reading (paper I). In extreme cases the unperfused (or very poorly perfused) regions covered more than 50% of a coronal brain section. Typically, the no-flow areas were located in the central parts of the rostral forebrain, mainly in the striatum, hippocampus,

amygdala, and thalamus. Extensions into adjacent cortical structures often appeared as wedge-shaped perfusion defects. In some instances no-flow spots were observed in the brain stem and the cerebellum. We note that these perfusion defects are similar in distribution to those described by Cantu and Ames (1969). In many regions the intrastructural heterogeneity made calculations of mean flow rates difficult or impossible.

In perfused parts of the brains hyperemia was observed in most structures. However, there were considerable interanimal variations, particularly in structures prone to develop no-reflow areas. In these, differences in reflow also often occurred between the two sides in individual brains. The importance of an adequate reperfusion pressure has been mentioned above. Peak blood pressure values during the first 5 min of reirculation varied among the animals between 140 and 190 mm Hg. Notably, the two animals with the least pronounced no-reflow had had the highest blood pressure peaks.

Experiments with shorter periods of compression ischemia were performed to establish the shortest insult required to elicit no-reflow. Perfusion defects were not seen after a shortlasting (2-3 sec) increase of the intracranial pressure above the blood pressure, neither after 5 min of compression ischemia, but after a 10 min ischemic period typical flow defects occurred. Measurement of LCBF after 30 min of compression ischemia showed no-flow areas, more extensive than those following the shorter ischemic periods.

Following 15 min of four-vessel-occlusion the autoradiograms revealed a no-reflow phenomenon in 4 of 6 animals, with a distribution similar to that observed following compression ischemia. Thus, we could exclude that the occurrence of postischemic perfusion defects is peculiar to the CSF compression model.

Animals subjected to 15 min of incomplete ischemia did not exhibit any initial postischemic perfusion defects. Rather, hyperemia prevailed in all structures examined and flow rates of 200-400% of control were recorded. CBF was homogenous within the structures examined.

Indomethacin and postischemic perfusion impairments

In a recent publication Hallenbeck and Furlow (1979) reported that pretreatment with the cyclo-oxygenase inhibitor indomethacin prevented impairment of postischemic flow rates in dogs subjected to

35 min of CSF compression ischemia. They measured local CBF 30 min after restoration of perfusion pressure. In a subsequent study from the same group the factor VIII/von Willebrand factor protein was made responsible for postischemic perfusion disorders, and the authors argued that progressive impairment of microvascular perfusion is set into motion by a process caused by brain tissue damage during ischemia (Hallenbeck et al. 1981). In view of these results we deemed it justified to examine the effect of cyclo-oxygenase inhibition in our models. Since the Hallenbeck group advocated an effect on secondary perfusion impairments we first studied the effect of pretreatment with indomethacin on delayed hypoperfusion following incomplete ischemia but observed very little effect on the 60 min recovery values (see below). In the light of our observation that no-reflow areas following 30 min of compression ischemia may persist for 90 min after restoration of perfusion pressure (below), we concluded that the circulatory impairments observed by the Hallenbeck group after 30 min of recirculation following 35 min of complete CSF compression ischemia should be considered as a lingering no-reflow phenomenon rather than a secondary deterioration of CBF. Consequently, we subjected indomethacin-injected animals to 15 min of complete compression ischemia and measured lCBF after 5 min of recirculation. The results showed that, although perfusion defects were observed also in indomethacin-injected animals, they were less extensive, and flow rates in perfused parts of the brain were much higher than in uninjected animals. A similar enhancement of immediate reflow was observed following incomplete ischemia, provided that the structures in question had been severely ischemic. We consider our results to be in line with those reported by Hallenbeck and Furlow (1979) although our interpretation may be different. It should be noted that the model used by the Hallenbeck group differs from ours, since they induced ischemia by elevating the intracranial pressure to the level of mean arterial blood pressure, but not above.

Reactive hyperemia and lactacidosis

It is generally assumed that postischemic hyperemia reflects vasoparalysis, possibly related to tissue acidosis. We observed that, following incomplete ischemia, hyperemia seemed to be more pronounced in the structures that had been only moderately ischemic than in those exposed to severe ischemia (paper II, fig.5). Since the potential for

lactate accumulation in the brain tissue is greater in the former, we speculated that a correlation may exist between the degree of tissue lactic acidosis incurred by ischemia and the level of hyperemia. In indomethacin-injected animals, though, the difference between structures with different degrees of previous ischemia is less obvious (paper III, fig.3), and if the effect of the anesthetic procedure is taken into account and the comparison is made with control values obtained in unanaesthetized animals, the difference is abolished (paper III, fig.8). Since we could show that treatment with indomethacin has no effect on blood glucose or cortical tissue lactate levels during and after ischemia, our results do not support the assumption that the level of postischemic hyperemia is related to the degree of lactate accumulation in the brain tissue.

Selective vulnerability

One of our objectives was to explore whether or not vulnerable regions in the brain show special characteristics in their postischemic vascular response. Following ischemia, cerebral cortex, hippocampus, and caudoputamen have been claimed to be particularly vulnerable (Brierley et al. 1975, Pulsinelli et al. 1982b). The only obvious correlation between these findings and the present results is that these structures, at least following complete ischemia, either show absence of hyperemia or an overt no-reflow phenomenon.

II. SIXTY MIN RECOVERY - delayed hypoperfusion

In general, our results from lCBF measurements after 60 min of recovery confirmed many previously reported data. Complete ischemia of 15 min duration, whether induced by CSF compression or by four-vessel-occlusion, and also incomplete ischemia of 15 min duration, are followed by a secondary reduction of flow with values averaging 50% of control. However, the autoradiographic technique used made it possible to obtain novel information on several aspects. First, following complete ischemia, the autoradiographic pictures showed that the perfusion defects had disappeared, and there was a much greater homogeneity within the structures than in the early recovery period. Second, and most important, there was a pronounced interstructural heterogeneity in spite of the uniform ischemic insult. Two structures had flow rates of less than 20% of control (frontal and auditory cortex) and two others of more than 80% of control (cerebellum and

inferior colliculus). Third, between 60 and 90 min of recovery some further reduction in LCBF occurred, most marked in structures in which no-reflow areas had been observed initially. This possibly reflects the fact that hyperemia is postponed in these structures. After 90 min of recovery the homogeneity within structures was even more pronounced than after 60 min.

The percentage reduction of LCBF 60 min after four-vessel-occlusion was, in the majority of structures, larger than after compression ischemia. However, the results were similar in the sense that CBF was homogenous within but exhibited marked differences between structures, with very low flow rates in the cerebral cortex and values close to normal in the cerebellum. The difference between the two models may in part be due to hemodilution occurring in compression ischemia.

Predictably, flow rates varied considerably between structures following incomplete ischemia, since different brain regions had been exposed to ischemia of different severity. Mean flow rates in structures that had been severely ischemic were even lower than following complete ischemia. The reduction was marked in frontal, auditory, sensorimotor, and parietal cortices (13, 16, 20, and 20% of control, respectively). A number of structures had fairly high flow rates, exceeding 40% of control (globus pallidus, hypothalamus, and habenula), in spite of the fact that ischemic flow rates in these regions had been below 20% of control.

Mean flow rates after 60 min of recovery were higher in fasted than in fed animals in the majority of structures. The difference was statistically significant in 10 of 24 structures examined. Although it seems that food withdrawal did enhance recovery flow rates, especially in regions with very low ischemic perfusion, there was still considerable hypoperfusion in fasted animals. This is shown in fig. 6, (paper II) in which the structures are listed, from top to bottom, in the order of severity of the preceeding ischemia. Although the results presented in this figure suggest a relationship between ischemic flow rates and the degree of delayed hypoperfusion, it must be recalled that interstructural differences in recovery CBF values existed also in complete ischemia. We must conclude, therefore, that the phenomenon of delayed hypoperfusion is related both to the severity of the preceeding ischemia, and to undefined vascular and/or

metabolic characteristics of different brain structures.

It was mentioned above that we have examined the effect of pretreatment with indomethacin on the 60 min recovery flow rates. The results presented in fig. 5 (paper III) show that indomethacin seemed to enhance recirculation in structures whose flow rates were markedly reduced during ischemia. Thus in all such structures except one (sensorimotor cortex) mean flow rates were higher in indomethacin-treated animals than in uninjected ones. However, this suggested relationship was lost in fasted animals (fig. 6, paper III). In these, indomethacin seemed to reduce CBF in a relatively uniform way (see also fig. 7, paper III). In structures with moderate reduction of CBF during ischemia and well preserved recovery flow rates (e.g. cerebellum and red nucleus, fig. 5, paper III), indomethacin treatment reduced CBF values. This is probably related to the well known effect of indomethacin on resting flow rates (Pickard and MacKenzie 1973, Sakabe and Siesjö 1979, Dahlgren et al. 1981b).

Thus, although the drug somewhat ameliorated delayed hypoperfusion in fed animals, the results obtained in fasted animals show that indomethacin does not generally improve postischemic perfusion. Obviously, delayed hypoperfusion must be due to other mechanisms than those related to enhanced cyclo-oxygenase activity. Our conclusion is that the beneficial effect of indomethacin on postischemic circulatory disturbances manifests itself primarily by enhancing blood flow in the immediate recirculation period.

Reactivity of the cerebral vessels during delayed hypoperfusion.

It was mentioned in the introduction that the cerebral vessels seem to be unreactive during the postischemic period dominated by delayed hypoperfusion. Vasoactive drugs fail to enhance CBF, and if the ischemic period is of sufficient duration hypercapnia does not increase CBF (Hossmann et al. 1973, Nemoto et al. 1975, Miller et al. 1980a). All these observations were made in experiments with models of complete ischemia. Hossmann et al. (1973) and Nemoto et al. (1975) measured global CBF only. Miller et al. (1980b) measured cortical CBF with a hydrogen clearance technique at multiple cortical sites in the recovery period following 5 min of complete ischemia. It may be concluded from the results of these three studies that CO₂ reactivity is attenuated after 3.5 min of ischemia, partially preserved after 5

min, but lost if the ischemia is prolonged to 10 min , or more.

We tested the CO₂ responsiveness of the cerebral vessels in the 60 min recovery period with a short exposure (2-3 min) to 10% CO₂. Following complete ischemia we did this to explore whether brain structures, subjected to a uniform degree of ischemia, show differences in the reactivity. The results showed that there were indeed pronounced interstructural variations in CO₂ responsiveness. First, in six structures (auditory, sensorimotor, and visual cortex, as well as caudoputamen, hippocampus, and globus pallidus) hypercapnia had no significant effect on CBF. Second, in 10 structures hypercapnia increased CBF to between 80 and 200% of normocapnic, nonischemic control. Third, two structures (red nucleus and cerebellum) had normal CO₂ responsiveness. Comparison was made with results obtained in a group of control animals exposed to 5-6 min of hypercapnia before measurement of lCBF. The results demonstrate that, even though all brain structures were subjected to complete ischemia of identical duration (with a certain reservation for the consequences of no-reflow, though), the vascular reactivity varied between complete loss and complete preservation of CO₂ responsiveness.

During incomplete ischemia of the present type CBF in different structures varied between virtually complete cessation of flow (e.g. the cerebral cortices) and a moderate reduction of flow (e.g. inferior colliculus and cerebellum). The model lends itself, therefore, to studies of relationships between the severity of the preceding ischemia and the loss/preservation of CO₂ reactivity. However, the most striking effect of hypercapnia during delayed hypoperfusion in this model was a marked intrastructural heterogeneity which was observed in 5 structures (parietal, auditory, frontal, sensorimotor, and visual cortices). The autoradiograms from 5 of 6 animals showed such heterogenities and, in these, fairly well delimited hyperemic areas were mixed with zones of low flow rates (fig.3 B, paper IV). When flow rates were calculated it was found that in all these cortical areas CO₂ responsiveness was markedly attenuated, in the whole structure or in part of it, in at least 4 of 6 experiments. In the hyperemic zones some values approached hypercapnic control values. In these areas, therefore, CO₂ responsiveness was relatively well preserved although CBF during ischemia must have been below 5% of control.

The results suggest a rough relationship between the severity

of the preceeding ischemia and the degree of reduction of CO₂ responsiveness (fig. 4, paper IV). However, two findings demonstrate that this relationship is not a tight one. First, the nucleus accumbens and the septal nucleus showed a strikingly low response. Second, the results obtained in complete ischemia (above) demonstrate that other factors than the degree of ischemia must at least partly be responsible for the loss/preservation of CO₂ reactivity.

The results of experiments in which the animals were exposed to hypoxia (PO₂ about 25 mm Hg) after 60 min of recovery can be summarized as follows. The response of the cerebral vessel to hypoxia was attenuated in most but not abolished in any of the structures examined. In general the response to hypoxia was better preserved than that to hypercapnia. Reactivity was similar following complete and incomplete ischemia. Intrastructural inhomogeneities resembling those seen during hypercapnia following incomplete ischemia were never observed, but, as in hypercapnia, there were pronounced interstructural variations with considerable increases in flow rates e.g. in the cerebellum (fig.6 paper IV).

The present results make it necessary to revise current concepts of cerebrovascular reactivity during postischemic recirculation, notably in the period of delayed hypoperfusion. Our results demonstrate that the CO₂ responsiveness differs considerably between regions, with variations between complete loss and complete preservation of the response. Another major result emerging from this study is that the reactivity to hypoxia is even better preserved. This result indicates that the cerebrovascular responses to hypercapnia and hypoxia are due to mechanisms that are at least in part dissimilar, mechanisms that are also differently affected by the ischemia. In view of these results it seems justified to redirect studies on the relationships between metabolic, circulatory, and structural sequelae of cerebral ischemia to the regional or local level.

CONCLUSIONS

The results obtained in these studies provide the following answers to the questions posed in the introduction.

No-reflow phenomenon. Unequivocal proof has been presented that complete ischemia of whatever cause carries a potential of inducing a

no-reflow phenomenon, probably because non-perfused vessels require a relatively high opening pressure. Obviously, this phenomenon involves an extension of the period of ischemia of affected tissue. The findings emphasize the therapeutic advantage of the "vascular flush" induced by full restoration of the postischemic perfusion pressure.

Regional differences in CBF during delayed hypoperfusion. One of the principal findings of the present study was the pronounced variability in reflow between structures following complete ischemia. Extreme variations between below 20% of control in some cerebral cortical structures and flow rates close to normal values in, e.g., the cerebellum were observed.

Nutritional state of the animals prior to ischemia. The results indicate that fasting is associated with higher local CBF values in animals allowed 60 min of recovery following 15 min of incomplete ischemia.

Reactivity of cerebral vessels following ischemia. Current views on postischemic cerebrovascular unreactivity should be revised. The results show a pronounced interstructural variability in CO₂ reactivity of cerebral vessels during delayed hypoperfusion, with extreme variations between complete loss and complete preservation of CO₂ responsiveness. The response to hypoxia is even better preserved than that to hypercapnia.

Effect of indomethacin. Pretreatment with indomethacin enhanced post-ischemic brain perfusion by reducing the extent of no-reflow areas and by increasing CBF in perfused regions in the early recovery phase. However, the drug failed to ameliorate the delayed hypoperfusion.

Selectively vulnerable regions. The only obvious correlation between previous observations of selectively vulnerable brain regions was that such areas (hippocampus, cerebral cortices, caudoputamen, and thalamus) showed little evidence of hyperemia and were prone to develop a no-reflow phenomenon in the immediate postischemic period.

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I

Local Cerebral Blood Flow in the Recovery Period
following Complete Cerebral Ischemia in the Rat

by

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Abstract

This study examines reflow patterns in the recirculation period following complete, global ischemia. CBF compression ischemia was induced in ventilated rats for 5-30 min, and local CBF was measured autoradiographically after 5, 60, and 90 min of recirculation. Ischemia of 15 min duration was induced by four vessel occlusion combined with arterial hypotension in two additional groups, with recovery periods of 5 or 60 min.

In the immediate recirculation period (5 min), following 15 min of ischemia, local CBF was markedly heterogenous. Thus, whereas most structures gave clear evidence of "reactive hyperemia" others showed perfusion defects of the "no-reflow" type. Typically these defects affected the striatum, thalamus, and hippocampus, as well as frontal, sensorimotor, and parietal cortex. Areas of no-reflow appeared after 10 min, they were more extensive after 15 min, and occupied a major part of the brain after 30 min of ischemia.

When recirculation was instituted for 60 or 90 min, following 15 min of ischemia, flow returned in previously unperfused areas. However, a delayed hypoperfusion developed, which differed widely between structures (range of CBF values 20-80 per cent of control). When the ischemic period was prolonged to 30 min, some perfusion defects remained even after 90 min of recirculation.

INTRODUCTION

Results reported during the last 10-15 years have characterized two types of postischemic circulatory disturbances. One of these, the so called no-reflow phenomenon, was originally described by Ames et al. (1968), who found areas of impaired perfusion when rabbit brains were perfused with carbon black at the end of ischemic periods of 5-7 min, or longer. As summarized in later publications from that laboratory (see Fischer 1973, Fischer et al. 1977) these postischemic perfusion defects have the following chief characteristics. First, with short periods of ischemia the defects mainly involved the basal ganglia, thalamus, and brain stem but, with longer periods, they occupied other structures as well, including the cerebral cortex. Second, the size of the perfusion defects diminished when a period of natural reperfusion preceded the carbon black perfusion. Third, the amount of perfused tissue increased when hemodilution was induced prior to ischemia, and when the perfusion pressure was increased. Since the no-reflow phenomenon seemed characteristic of complete ischemia, and notably of that allowing blood to stagnate in the vasculature, it was assumed to be caused by erythrocyte stasis and increased blood viscosity in vessels whose lumina were narrowed due to perivascular glial swelling. However, a recent article from the same laboratory suggests that perfusion defects are minimal if reperfusion occurs at normal perfusion pressures, even if ischemia is sustained for 15 min (Fischer et al. 1979). It is difficult to judge, therefore, whether the no-reflow phenomenon, as originally described, is an experimental artefact or a pathophysiological entity.

The no-reflow phenomenon has been noted in other species as well, albeit with somewhat different distribution (Ginsberg and Myers 1972, Wade et al. 1975, Lin and Korman 1977). Unfortunately, none of these studies give information on natural reperfusion or provide data on actual blood flow rates. Instead, studies in which overall or regional CBF has been measured have focussed interest on another type of postischemic circulatory disturbance. In this, recirculation typically leads to a transient increase in CBF ("reactive hyperemia", cf Häggendal et al. 1970), later to be followed by a secondary ("delayed") hypoperfusion (Zimmer et al. 1971, Hossmann et al. 1973, Snyder et al. 1975, Hossmann 1977, Tagaki et al. 1977, Nordström and

Rehncrona 1977, Steen et al. 1978, Kofke et al. 1979, Levy et al. 1979, Miller et al. 1980, Jackson et al. 1981, Pulsinelli et al. 1982b). We note that this time course is opposite to that described for the no-reflow phenomenon. In view of these results, it is tempting to conclude that if natural recirculation occurs at a high perfusion pressure, a true no-reflow phenomenon does not occur; rather, post-ischemic circulatory disturbances are dominated by delayed hypoperfusion (Hossmann 1977). This conclusion is corroborated by studies in which measurements of local CBF (with autoradiographic techniques) failed to disclose perfusion defects in the immediate postischemic period (Marshall et al. 1975, Levy et al. 1979, Pulsinelli et al. 1982a), but which clearly documented hypoperfusion in later stages of the recirculation period (Ginsberg et al. 1978, Norström and Rehncrona 1977, Hallenbeck and Bradley 1977, Hallenbeck and Furlow 1979, Levy et al. 1979, Pulsinelli et al. 1982 a).

The pathogenic importance, if any, of these postischemic changes in CBF is not known. However, evidence exists that the initial hyperemia is a prerequisite for functional recovery, at least following long periods of ischemia (Hossmann et al. 1973). Furthermore, it has been speculated that the delayed hypoperfusion may add a secondary ischemic insult to the tissue, especially if the reduction in CBF is not matched by a corresponding lowering of metabolic rate (see Snyder et al. 1975, Hossmann et al. 1976, Nordström and Rehncrona 1977, Levy and Duffy 1977, Kofke et al. 1979, Levy et al. 1979, Pulsinelli et al. 1982 a and b).

It has been clearly documented that any cell damage incurred after cerebral ischemia shows a preferential localization to certain selectively vulnerable regions. The question arises, therefore, if such regions (e.g. the hippocampus, caudoputamen, and cerebral cortex) shows a less extensive postischemic hyperemia, and a more pronounced secondary hypoperfusion, than less vulnerable regions. Since available results do not permit conclusions to be drawn on this matter, we decided to study recirculation following a standardized ischemic insult. Our objectives made it necessary to choose an ischemic insult that was uniform throughout the brain, and that allowed rapid resumption of a normal perfusion pressure in the immediate recirculation period. Furthermore, we wished to avoid the metabolic depression exerted by barbiturates and the potential pitfalls of a slowly

deteriorating circulation, as this may occur following long ischemic periods and/or with excessive cellular acidosis. For all these reasons, we induced compression ischemia of 15 min duration in rats maintained ventilated on nitrous oxide:oxygen (70:30), and measured CBF autoradiographically after 5, 60, and 90 min. In order to study whether the results were generally valid, or peculiar to CSF compression ischemia, other animals were exposed to four-vessel-occlusion of similar duration, with recovery periods of 5 and 60 min.

METHODS

Operative, sampling, and analytical techniques

All experiments were performed on male Wistar rats (350-400 g) of a S.P.F. strain (Møllegaard Avlslaboratorium, Copenhagen), that had free access to tap water and rat pellets until operation. After induction of anesthesia with 3% halothane the rats were tracheotomized and artificially ventilated with a mixture of 0.7% halothane in 30% O₂ and 70% N₂O during the subsequent operation. The femoral arteries, femoral veins, and one brachial artery were cannulated for blood sampling, continuous blood pressure recording, infusions, and injections. Goldplated copper screws were inserted into the skull bone for EEG recordings. The animals were paralyzed with tubocurarine chloride (2 mg.kg⁻¹ i.v.) and given heparin (100 I.U. i.v.). After completion of the surgical procedure the halothane supply was discontinued and ventilation was adjusted to give arterial oxygen (PaO₂) and carbon dioxide (PaCO₂) tensions of about 100 and 33-40 mm Hg, respectively. The animals were then allowed a steady state period of 30 min before induction of ischemia.

Arterial blood was sampled anaerobically in glass capillaries for blood gas determinations. PaO₂, PaCO₂ and pH were measured with microelectrodes (Eschweiler and Co., Kiel, and Radiometer, Copenhagen), operated at 37°C, with due corrections for the body temperature. Analysis of whole blood concentrations of glucose, lactate, and pyruvate were performed after sampling of arterial blood directly into liquid nitrogen.

Statistical comparison was performed using Student's t-test, $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$

Reversible complete compression ischemia

Ischemia was induced as described by Ljunggren et al. (1974) and Nordström et al. (1978). The intracranial pressure was momentarily increased to at least 40 mm Hg above the systolic pressure by infusing an artificial CSF solution into the cisterna magna through a double-barrelled needle, which allowed the intracranial pressure to be recorded simultaneously. The hypertensive reaction to increased intracranial pressure was minimized by i.v. infusion of Arfonad^(R) (Tri-methaphan-camphor-sulphonate), often supplemented by arterial bleeding. During the ischemia a fall in the intracranial temperature was prevented by keeping a heating bulb at a predetermined distance from the head of the animal. We restored the cerebral perfusion pressure by discontinuing the cisternal infusion and normalizing the blood pressure.

Reversible complete four-vessel-occlusion ischemia

The upper part of the sternum was removed to allow approach to the anterior aspect of the caudal part of the cervical column. The transverse process of the 6th vertebra and the vertebral arteries and veins were identified on both sides. The vagus and sympathetic trunks were separated from the common carotid arteries. A No 1 nylon thread was applied behind the oesophagus but in front of the common carotid arteries (leaving the nerve trunks outside) and, with a needle, it was then drawn through the paravertebral muscles bilaterally at the level C6-C7. A length of rubber tubing was threaded on the ligature where it rested on the common carotid arteries to prevent injury to the vessels walls during compression. Some fibrous and muscle tissue was left between the thread and the vertebral arteries, for the same purpose. Four-vessel-occlusion was accomplished by tying the thread in the back of the animal. To compress collaterals a cotton band was tied hard around the neck above the ligature. The trachea was kept outside the cotton band. Before vessel occlusion was started blood pressure was lowered to about 50 mm Hg by i.v. infusion of Arfonad^(R) and kept at that level during the ischemia. If necessary, Arfonad infusion was supplemented by arterial bleeding. After 15 min of ischemia we restored circulation in the brain by releasing the ties and normalizing the blood pressure with blood infusion. During recirculation the

ventilation volume was increased to counteract an increase in the PaCO_2 and small doses of sodium bicarbonate were injected i.v. to compensate for plasma acidosis.

The efficacy of the four-vessel-occlusion was tested in two ways. In two animals LCBF was measured during ischemia with the autoradiographic technique described below. The films showed no activity in the brains. In 6 animals we examined the accumulation of lactic acid in the ischemic brains. Following 15 min of four-vessel-occlusion plus hypotension the brains were frozen in situ with the technique described by Pontén et al. (1973). The brains were then chiselled out and extracts from cerebral cortex were subsequently analyzed for contents of labile cerebral metabolites. Four sham-operated animals were used as controls. The results showed that, at the end of ischemia, the content of phosphocreatine had decreased to $0.18 \pm 0.05 \mu\text{mol.g}^{-1}$ and that of ATP to $0.08 \pm 0.02 \mu\text{mol.g}^{-1}$. Compared with the shamoperated animals the lactic acid concentration of ischemic brain tissue had increased by $13.1 \pm 0.7 \mu\text{mol.g}^{-1}$ (from 2.4 to $15.5 \mu\text{mol.g}^{-1}$) while the increase that could be expected to occur from the preischemic (control animals) contents of glucose and glycogen (lactate = 2 glucose + 2 glycogen) was calculated to $14.0 \pm 0.2 \mu\text{mol.g}^{-1}$. The results demonstrate, therefore, that the ischemia was complete, at least in the examined parts of the ischemic brains.

Measurement of local CBF (LCBF)

Local CBF was measured with the technique described by Landau et al. (1955) as modified by Sakurada et al. (1978). Details of procedure have been given in previous communications (Abdul-Ranman et al. 1979, Ingvar et al. 1981). In summary, 30-50 μCi of ^{14}C -iodoantipyrine, dispensed in 0.4 ml of Krebs-Hensleit solution, was infused i.v. at a constant rate for 30 sec (45 sec if a reduced LCBF was expected). Blood samples were obtained from the brachial artery catheter before and every third sec (5th sec) during the infusion. At 30 sec (45 sec) the animal was decapitated. The brain was then removed from the skull (within 2 min) and immediately immersed in isopentane chilled to -55 to 65°C . The ^{14}C activity in brain tissue (per unit mass) was evaluated by autoradiography. Slices of brain (20 μm) were cut in a Leitz sledge microtome at -20°C and applied to an X-ray film which was then exposed for one week. A series of calibrated ^{14}C -stan-

dards was applied to each film together with the brain slices. The film was then evaluated manually with a densitometer (McBeth, 1 mm aperture).

Local CBF was calculated as described by Landau et al. (1955). With the sampling modification used it was not possible to determine lCBF accurately at all flow rates. Structures whose activity was less than the lowest ^{14}C -standard were assigned flow values corresponding to that of the lowest standard. (This means that mean lCBF in some structures with reduced flow was slightly overestimated.)

Experimental groups

Local CBF during recovery following complete compression ischemia of 15 min duration was evaluated in three groups, each comprising 6 animals. Recirculation time after the end of ischemia was 5, 60, and 90 min, respectively.

To study the influence of the duration of ischemia on the occurrence of "no-reflow", lCBF was also measured in the early recovery phase (5 min) following ischemia of 5 and 10 min duration, with two animals in each group, and in 3 animals after 5 min of recirculation following a shortlasting (2-5 sec) increase of the intracranial pressure above the blood pressure.

The possible effect of prolongation of the ischemia on the rate of disappearance of "no-reflow" areas was evaluated in 6 animals in which lCBF was measured following complete compression ischemia of 30 min duration with recovery periods of 5, 30, and 90 min, with two animals in each group.

In two groups of 6 animals each lCBF was studied after 5 and 60 min of recirculation following 15 min of complete four-vessel-occlusion ischemia.

Control values for lCBF were obtained from animals kept artificially ventilated for periods corresponding to the duration of the experiments. Anesthesia, as well as operative and sampling techniques were the same as in the experimental groups.

R E S U L T S

Physiological parameters

Table 1 shows the physiological parameters in the main groups included in the present material. The ischemia did not alter plasma pH. Changes in body temperature, blood pressure, PCO₂ and PO₂ were small and it seems less likely that they should have influenced ICBF in the recovery periods. However, it should be noted that some animals had mean arterial blood pressures below 120 mm Hg after 60 min of recovery (minimum value 110 mm Hg). Furthermore, after 5 min of recovery following four-vessel-occlusion, the arterial PCO₂ was increased by about 6 mm Hg and after 90 min of recovery following compression ischemia PCO₂ was reduced by about 5 mm Hg. Physiological

Table 1. Physiological parameters in control animals and in animals subjected to either complete compression ischemia or complete four-vessel-occlusion ischemia of 15 min duration.

Experimental groups	Temp. °C	MABP mm Hg	PaO ₂ mm Hg	PaCO ₂ mm Hg	pH	n
Control animals	37.1±0.1	152±2	100±5	36.8±0.8	7.41±0.01	7
Compr.isch.Rec.5'						
Preisch	37.3±0.1	143±6	115±5	38.0±0.5	7.40±0.02	6
Recirc. 5'	36.7±0.1	144±8	112±11	38.8±2.2	7.41±0.02	
Compr.isch.Rec.60'						
Preisch	37.0±0.2	140±4	102±3	37.1±1.1	7.44±0.01	6
Recirc. 15'	37.4±0.2	139±2	109±9	32.0±1.2	7.46±0.01	5
Recirc. 60'	37.4±0.2	125±4	121±7	35.3±1.2	7.42±0.01	6
Compr.isch.Rec.90'						
Preisch.	37.2±0.2	142±7	105±3	38.7±0.8	7.41±0.01	6
Recirc. 90'	37.3±0.2	130±5	130±7	33.3±0.7	7.42±0.02	6
Four-vessel-occlu- sion. Recirc. 5'						
Preisch.	37.3±0.4	156±5	101±2	37.8±1.0	7.38±0.02	6
Recirc.5'	36.6±0.3	129±2	98±10	42.8±3.4	7.36±0.03	6
Four-vessel-occlu- sion. Recirc. 60'						
Preisch.	37.3±0.2	152±1	97±2	37.5±0.9	7.39±0.02	6
Recirc-15'	37.4±0.2	139±6	112±10	37.9±1.3	7.36±0.02	6
Recirc. 60'	37.2±0.1	132±2	127±3	39.0±1.4	7.34±0.02	6

The values are means + SEM. MABP= Mean arterial blood pressure.
n= number of animals examined.

parameters in animals not included in the table were similar to those obtained in the main groups (data not shown).

Perfusion pressures

Since reperfusion following ischemia depends on adequate perfusion pressure (see Introduction) the latter was carefully followed. Before cisternal infusion of mock CSF was begun, mean arterial pressure was reduced to about 90 mm Hg by i.v. infusion of Arfonad (fig. 1). Nevertheless, a hypertensive response usually occurred when

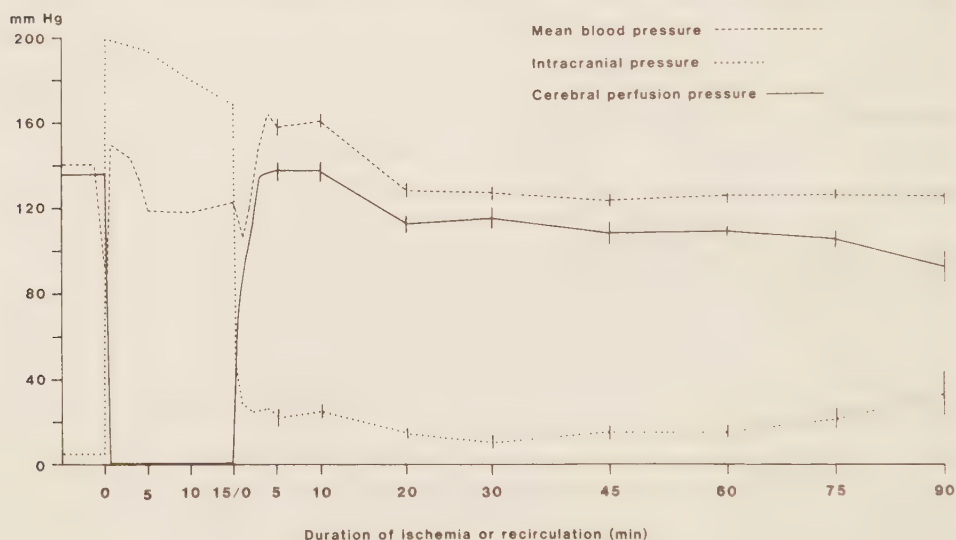


Fig. 1

Blood pressure, intracranial pressure, and cerebral perfusion pressure before, during, and following 15 min of complete compression ischemia. Values are means $\pm$ SEM.

the intracranial pressure was raised above 200 mm Hg. Arterial bleeding was often required to counteract this reaction. (Continued infusion of Arfonad had usually no effect in this situation.) When ischemia had lasted a few minutes the blood pressure tended to fall spontaneously. The intracranial pressure was then also allowed to decrease somewhat, but was always kept at least 30 mm Hg above the mean arterial pressure. This adjustment of the CSF pressure seemed to prevent an undesirable drop in the blood pressure and also to reduce the risk for development of pulmonary edema.

Interruption of the cisternal infusion elicited a shortlasting

hypotensive reaction, which was counteracted by i.v. infusion of blood. However, the blood pressure was usually normalized within 2 min and reached values above normal during the following minutes. Peak blood pressure values during the first 5 min of recirculation varied among the animals between 145 and 190 mm Hg.

The intracranial pressure fell abruptly when the cisternal infusion was discontinued, down to a point between 80 mm Hg and zero (average 45 mm Hg). During the following seconds it fell more slowly and within one min it attained a value between 50 mm Hg and zero. A small rise in the CSF pressure concomitant with the blood pressure peak was often observed. In 3 animals the cisterna magna pressure remained elevated, between 30 and 50 mm Hg, throughout the recirculation period. In the group of animals which were recirculated for 90 min there was a tendency towards a rise in the CSF pressure during the last 30 min of recirculation.

The mean arterial blood pressure before, during, and following four-vessel-occlusion is illustrated in fig. 2. Following release of

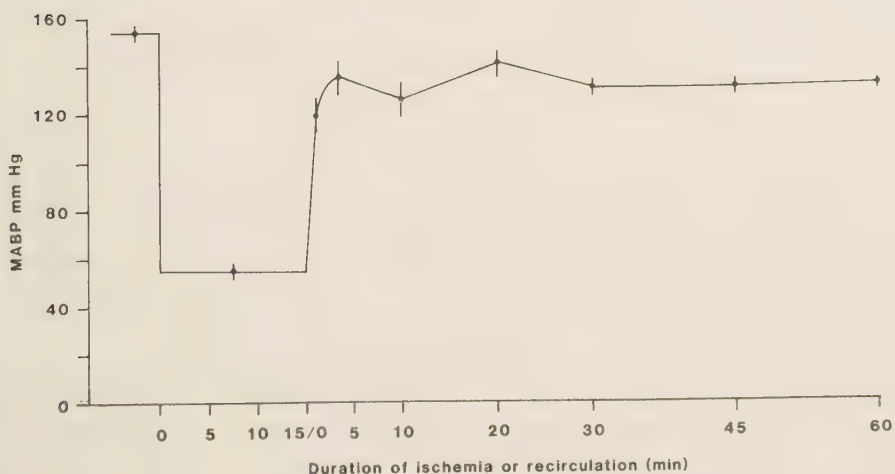


Fig. 2

Mean arterial blood pressure before, during, and following complete four-vessel-occlusion ischemia. Values are means $\pm$ SEM.

vascular occlusion, and reinfusion of blood, blood pressure rose to above 100 mm Hg within 1-2 min, and reached values of 130 mm Hg in about 3 min. If one takes into account that recirculation leads to a

transient increase in intracranial pressure to about 25 mm Hg (with a peak occurring after about 5 min, (see Snyder et al. 1975, Hossmann 1977) it seems permissible to conclude that restoration of cerebral perfusion pressure was relatively similar with the two ischemic models.

Cerebral blood flow

Table 2 lists lCBF values for 25 different structures. Enclosed are the control values and the values obtained after 60 and 90 min recirculation following 15 min of ischemia. Values obtained in the other groups could not be meaningfully tabulated due to large inter-animal variations and intrastructural inhomogeneities. In the following we will present the CBF data under two separate headings.

1. Recirculation following ischemia - hyperemia versus no-reflow

The purpose of the experiments reported under this heading was to establish if 15 min of complete ischemia is followed by generalized hyperemia, or if some structures show postischemic perfusion defects. Since such defects were found, it became of interest to assess the shortest period of ischemia which lead to loci of poorly perfused tissue. Furthermore, since perfusion defects following 15 min of ischemia had resolved after 60 min of reperfusion (see next section), we studied whether such reperfusion also occurred after 30 min of ischemia.

The autographic films from animals subjected to 15 min of compression ischemia, and allowed 5 min of recirculation, revealed the presence of flow defects (fig. 3). Densitometric readings verified that all 6 animals had no-flow areas of varying size and extension. In one animal there were only small spots of no-flow, 1-2 mm in diameter (fig. 3, A and B). A second animal exhibited somewhat larger no-flow patches (fig. 3, C and D). All the others had extensive confluent no-flow areas, in extreme cases covering more than 50 per cent of a coronal brain section (fig. 3, E and F). Typically, no-flow areas were located in the central parts of the rostral forebrain - mainly in the striatum, hippocampus, amygdala, and thalamus. In the cortex the most

TABLE 2. Local CBF in control animals and following 15 min of complete compression ischemia or four-vessel-occlusion ischemia.

	Controls n=7	Compr.isch.15' Recirc. 60'	Compr.isch.15' Recirc. 90'	Four-vessel-occl. isch. 15' Recirc. 60' n=6
Frontal cortex	2.03 $\pm$ 0.08	0.37 $\pm$ 0.08	0.26 $\pm$ 0.04	0.44 $\pm$ 0.06
Nucl. accumbens	1.50 $\pm$ 0.10	0.67 $\pm$ 0.07	0.61 $\pm$ 0.14	0.52 $\pm$ 0.06
Caudatoputamen	1.41 $\pm$ 0.09	0.94 $\pm$ 0.10	0.76 $\pm$ 0.12	0.64 $\pm$ 0.14
Globus pallidus	0.66 $\pm$ 0.07	0.51 $\pm$ 0.03	0.41 $\pm$ 0.06	0.36 $\pm$ 0.07
Subst. nigra	1.01 $\pm$ 0.07	0.55 $\pm$ 0.04	0.59 $\pm$ 0.04	0.73 $\pm$ 0.15
Red nucleus	1.45 $\pm$ 0.10	0.85 $\pm$ 0.04	0.69 $\pm$ 0.05	0.89 $\pm$ 0.09
Cerebellum	0.82 $\pm$ 0.06	0.78 $\pm$ 0.07	0.64 $\pm$ 0.08	0.63 $\pm$ 0.09
Sensorimotor cortex	1.98 $\pm$ 0.10	0.86 $\pm$ 0.07	0.67 $\pm$ 0.11	0.68 $\pm$ 0.07
Parietal cortex	1.89 $\pm$ 0.09	0.58 $\pm$ 0.06	0.50 $\pm$ 0.06	0.60 $\pm$ 0.07
Thalamus	1.63 $\pm$ 0.09	1.12 $\pm$ 0.06	0.86 $\pm$ 0.13	0.80 $\pm$ 0.13
Lat. thalamus	1.53 $\pm$ 0.09	0.92 $\pm$ 0.05	0.80 $\pm$ 0.12	0.71 $\pm$ 0.08
Visual cortex	1.52 $\pm$ 0.08	0.56 $\pm$ 0.06	0.44 $\pm$ 0.09	0.39 $\pm$ 0.06
Lat. geniculate body	1.40 $\pm$ 0.11	0.53 $\pm$ 0.05	0.51 $\pm$ 0.06	0.49 $\pm$ 0.05
Superior colliculus	1.48 $\pm$ 0.11	0.71 $\pm$ 0.06	0.70 $\pm$ 0.05	0.64 $\pm$ 0.08
Auditory cortex	3.12 $\pm$ 0.41	0.63 $\pm$ 0.08	0.47 $\pm$ 0.04	0.67 $\pm$ 0.12
Medial geniculate	1.82 $\pm$ 0.09	0.59 $\pm$ 0.06	0.57 $\pm$ 0.05	0.57 $\pm$ 0.07
Inferior colliculus	1.99 $\pm$ 0.09	1.55 $\pm$ 0.28	1.35 $\pm$ 0.11	1.2 $\pm$ 0.16
Piriform cortex	1.12 $\pm$ 0.10	0.41 $\pm$ 0.06	0.31 $\pm$ 0.04	0.31 $\pm$ 0.03
Cingulate cortex	1.33 $\pm$ 0.12	0.58 $\pm$ 0.12	0.50 $\pm$ 0.11	0.44 $\pm$ 0.07
Hippocampus	0.87 $\pm$ 0.07	0.62 $\pm$ 0.09	0.46 $\pm$ 0.07	0.45 $\pm$ 0.07
Amygdala	1.06 $\pm$ 0.09	0.60 $\pm$ 0.10	0.48 $\pm$ 0.08	0.31 $\pm$ 0.03
Septal nucleus	0.85 $\pm$ 0.06	0.42 $\pm$ 0.04	0.42 $\pm$ 0.05	0.31 $\pm$ 0.04
Habenula	1.64 $\pm$ 0.12	0.76 $\pm$ 0.04	0.69 $\pm$ 0.05	0.64 $\pm$ 0.10
Hypothalamus	1.04 $\pm$ 0.08	0.57 $\pm$ 0.04	0.58 $\pm$ 0.06	0.43 $\pm$ 0.06
Manillary body	1.83 $\pm$ 0.16	0.97 $\pm$ 0.10	0.73 $\pm$ 0.06	0.89 $\pm$ 0.10

Local CBF is expressed in $\text{ml.g}^{-1}.\text{min}^{-1}$.

characteristic finding was wedge-shaped flow defects beginning in the subcortical white and terminating in the intermediate layers of the the frontal, sensorimotor, and parietal cortices (fig. 3, D). Superficial layers of the cortex were seldom involved. In one animal no-flow patches were seen also in the brain stem and, in 3 animals, there were no-flow spots in the cerebellum.

Among factors that may influence the occurrence of a no-reflow phenomenon, postischemic perfusion pressure can be expected to be of special importance. As stated, peak blood pressure values during the first 5 min of recirculation varied among the animals between 145 and

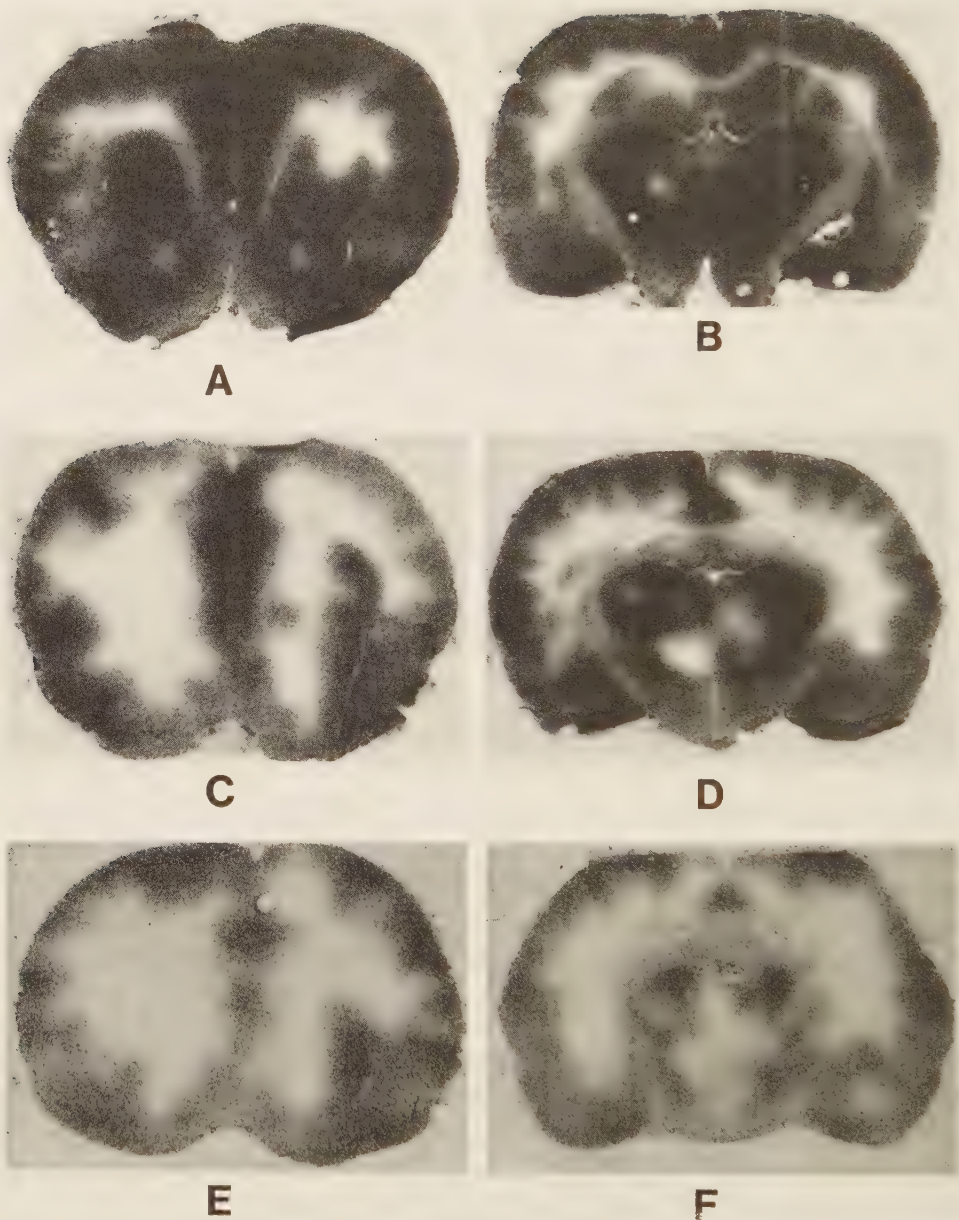


Fig. 3 Autoradiographic pictures from coronal sections of the caudoputamen/sensorimotor cortex region (left) and the thalamus/parietal cortex region (right), showing no-reflow phenomenon of slight (A and B), moderate (C and D), and severe (E and F) degrees following 15 min of complete compression ischemia and 5 min of recirculation.

190 mm Hg. Notably, the two animals with the least pronounced no-reflow had had the highest blood pressure peaks.

The results of fig. 3 emphasize the pronounced heterogeneity of lCBF both between animals, and within regions in the same animal. In order to allow graphic illustration of local differences in lCBF,

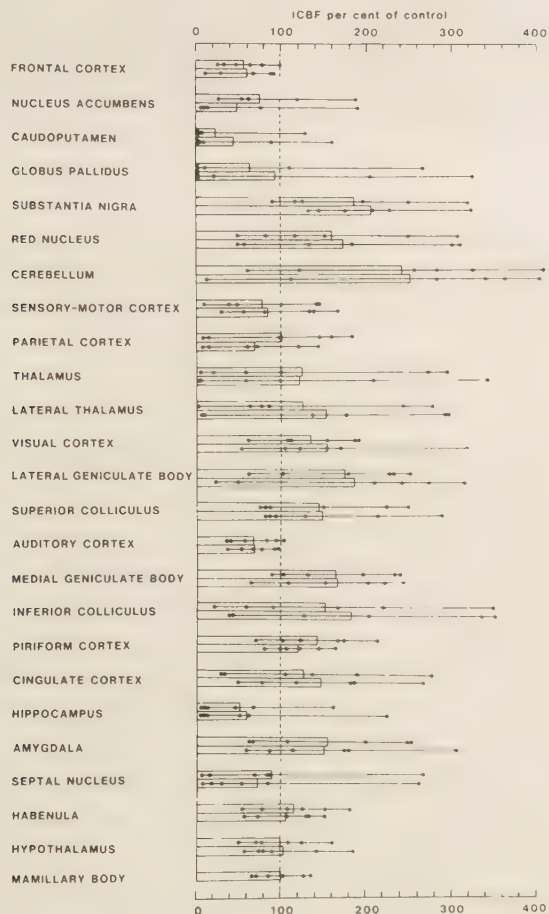
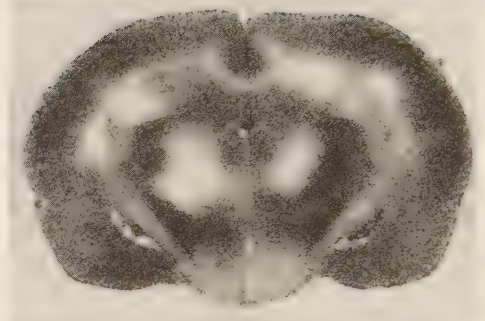


Fig. 4

Mean and individual lCBF values in per cent of control in 25 brain structures after 5 min of recirculation following 15 min of complete compression ischemia. Symmetric structures were evaluated on the two sides. For each structure values from the left hemisphere are found in the upper column, values from the right hemisphere in the lower column. The clusters at the bottom of the columns for the caudoputamen and globus pallidus represent 3 values at or close to zero in each column.



A



B

Fig. 5 Autoradiographic pictures from coronal sections of the caudoputamen/sensorimotor cortex region (A), and the thalamus/parietal cortex region (B), showing moderate degree of no-reflow phenomenon after 5 min of recirculation following 15 min of complete four-vessel-occlusion ischemia.

mean CBF values for the different structures were derived from at least 6 densitometric readings. Furthermore, to explore whether side differences could occur each symmetric structure was read on the two sides. The results (fig. 4) allow the following conclusions. First, although the mean values showed that hyperemia occurred in the majority of structures, large variations occurred between animals. Such variations were extreme in structures prone to develop a no-reflow phenomenon (cf. fig. 3). In these, differences in reflow also often occurred between the two sides of individual brains. Second, in some other structures most (or all) of the CBF values measured were below control (frontal and auditory cortex, hippocampus, and septal nucleus, see also sensorimotor and parietal cortex).

The results led to three questions. First, is the appearance of a true no-reflow phenomenon an experimental artefact, related to the ischemic model? Second, if this is not the case, what is the minimal experimental period required to elicit the phenomenon? Third, does the duration of ischemia influence the conditions for restoration of flow in the no-flow areas? In order to provide answers to the first question, six animals exposed to 15 min of four-vessel-occlusion (see methods) were studied. In four of these animals, areas of no-reflow were observed, with a distribution similar to that following

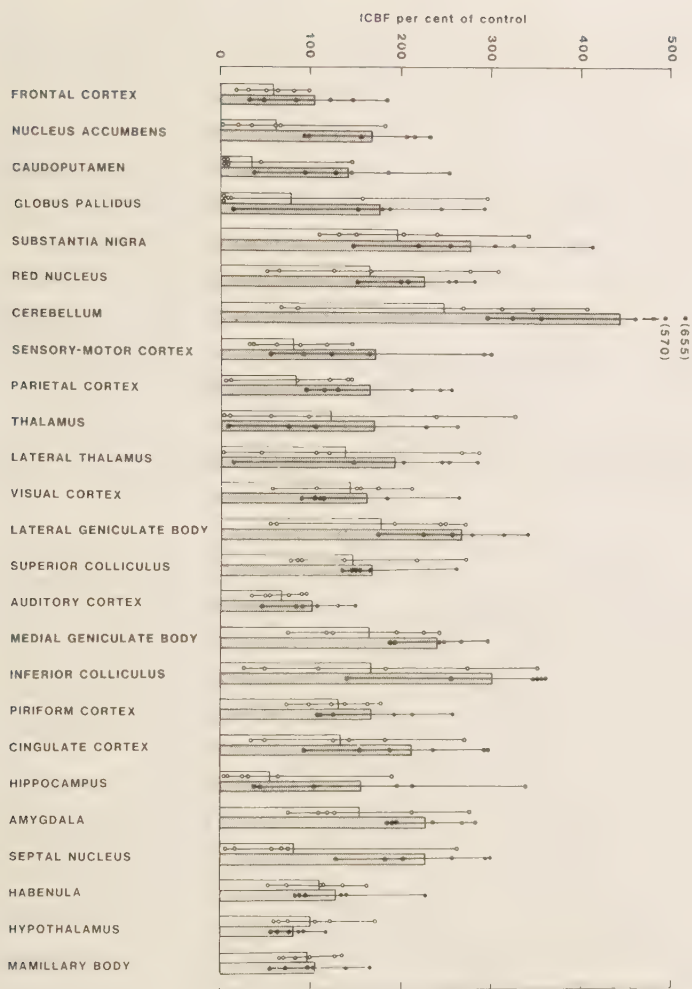


Fig. 6

Mean and individual ICBF values in per cent of control after 5 min of recirculation following 15 min of complete compression ischemia (open dots), and 15 min of complete four-vessel-occlusion ischemia (filled dots), respectively. In each group flow rate values from the two hemispheres were pooled. No-reflow occurred in both ischemic models (see text), but since unperfused areas were less extensive and never occupied a whole structure following vascular occlusion ischemia, mean flow rates were always above zero in that group.

compression ischemia, but in general less extensive and with comparatively little engagement of the cerebral cortices (fig. 5). We could exclude that no-reflow following four-vessel-occlusion was due to a general hindrance to recirculation. Thus, if one compares ICBF in all structures not affected by the no-reflow phenomenon following compression ischemia and four-vessel-occlusion, respectively, one finds that the latter model yielded ICBF values that appeared to exceed those observed after compression ischemia (fig.6).

To provide answers to the second question, reflow was studied in two animals after 5 and in another two after 10 min of compression ischemia. In addition, in order to explore whether or not the intracranial pressure per se affected reflow, 3 animals were subjected to only 2-5 sec increase in the intracranial pressure before 5 min recirculation was initiated.

Following 2-5 sec ischemia, ICBF was within the ranges of control values in 20 of 25 structures (data not shown). However, in auditory, frontal, parietal, sensorimotor, and visual cortex, mean ICBF after 5 min of recirculation was 48, 59, 67, 67, and 71 per cent of control, respectively. Thus, the increase in intracranial pressure per se neither induced hyperemia nor no-reflow but it reduced CBF in several cerebral cortical areas (see Discussion).

The results of ICBF measurements after 5 min of recovery following compression ischemia of 5 and 10 min duration, respectively, are shown in fig. 7. Five min ischemia was followed by moderate to marked hyperemia in about 50 per cent of the structures. Somewhat reduced flow rates were observed in frontal cortex, the geniculate bodies, hypothalamus and mamillary body. There were no no-reflow areas. After 10 min ischemia and 5 min recirculation there was hyperemia in the majority of structures. No-flow areas occurred, though, in frontal, sensorimotor, and parietal cortex, as well as in the caudoputamen, thalamus, inferior colliculus, and hippocampus. In these structures ICBF was extremely heterogenous.

The third question concerns the time of resolution of no-reflow. It will be shown below that the no-reflow areas observed after 5 min of recirculation, following 15 min of ischemia, had disappeared after 60 min of recirculation. Measurements of CBF following 30 min of compression ischemia showed large no-reflow areas after 5 min of recirculation, more extensive than after 15 min of ischemia but with

the same distribution (fig. 8). After 30 and 90 min of recovery large patches of no-flow persisted, especially in striatum and thalamus, albeit less pronounced than in the early recovery phase. Two further findings should be emphasized. First, although some cortical and subcortical areas showed loci of high flow rates, true hyperemia (i.e. an increase of CBF above normal) was not observed. Second, perfused regions had flow rates even lower than those given in table 2, see also below). Obviously, with such long ischemic periods under-perfusion of many brain structures may persist for at least an hour (see Discussion).

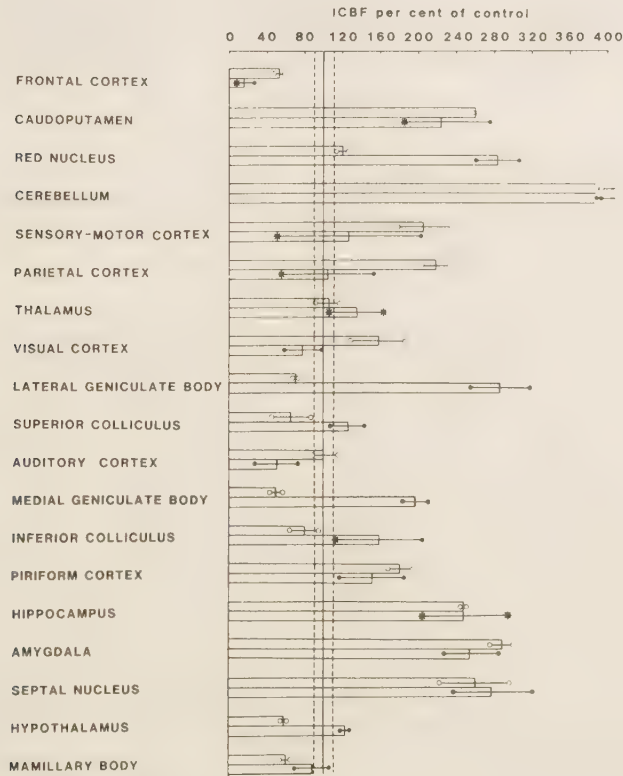


Fig. 7

Individual local CBF values in per cent of control after 5 min of recirculation following complete compression ischemia of 5 min (upper columns and open dots), and 10 min (lower columns and filled dots) duration, respectively.
 * indicates the occurrence of no-reflow phenomenon in the structure.

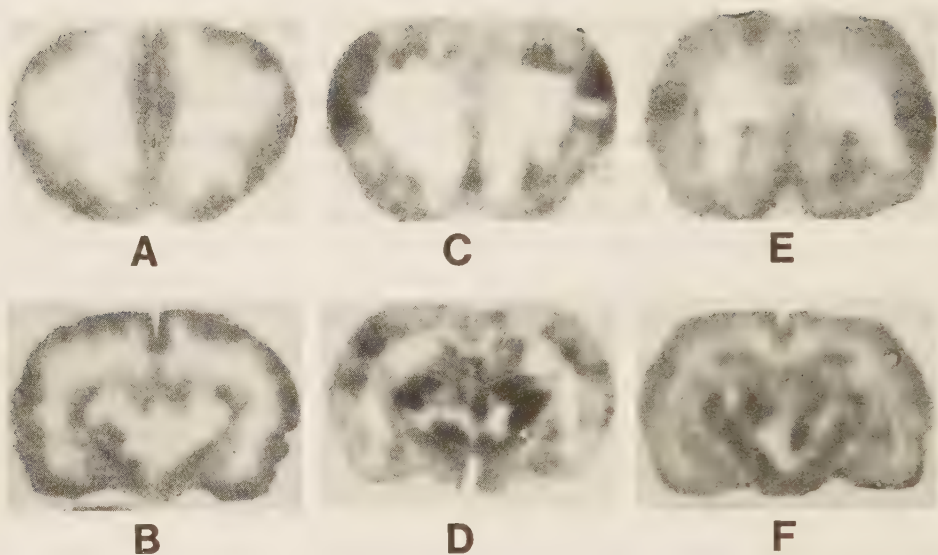


Fig. 8 Autoradiographic pictures from coronal sections of the caudoputamen/sensorimotor cortex region (upper) and the thalamus/parietal cortex region (lower) following 30 min of complete compression ischemia and recirculation of 5 (A and B), 30 (C and D), and 90 (E and F) min duration, respectively, showing extensive no-reflow phenomenon, remaining even after 90 min of recirculation.

2. Recirculation following ischemia - delayed hypoperfusion

In order to explore if regional differences occurred in the degree of delayed hypoperfusion (see Introduction) $lCBF$ was measured after 60 and 90 min of recirculation, following 15 min of compression ischemia. The autoradiographic pictures from brain sections after 60 min of recovery showed a much greater homogeneity than those obtained in the early recovery period. Thus, although some structures, especially the caudoputamen and the thalamus, appeared somewhat speckled, there were no no-reflow areas. After 90 min of recovery the homogeneity within structures was even more prominent. Since the densitometric readings confirmed the visual impression of homogeneity, mean values were calculated (see Table 2 above).

In fig. 9, values obtained after 60 and 90 min of recirculation have been given in per cent of control, together with statistical figures for differences between the 60 and 90 min groups. Furthermore, values for cerebral cortical structures have also been given in per cent of control values as obtained in awake, minimally restrained rats (see Discussion). The following results emerge. First,

although the mean lCBF values were usually lower in the 90 than in the 60 min group, the differences were not large. Therefore, it seems permissible to use the combined data to indicate the degree of delayed hypoperfusion. Second, in spite of the uniform ischemic insult, the percentage reduction in lCBF was heterogenous. For example, two structures had flow rates of less than 20 per cent of control (frontal and auditory cortex) and two others of more than 70 per cent of control (cerebellum and inferior colliculus). The reduction of lCBF in frontal and auditory cortex (to 12-20% of control) becomes less extreme, though, if values are calculated in per cent of awake control (20-30%). Third, relatively large differences in flow rates occurred between cortical structures (see frontal and sensorimotor cortex), and other "vulnerable" structures had relatively high CBF values (see caudoputamen and hippocampus).

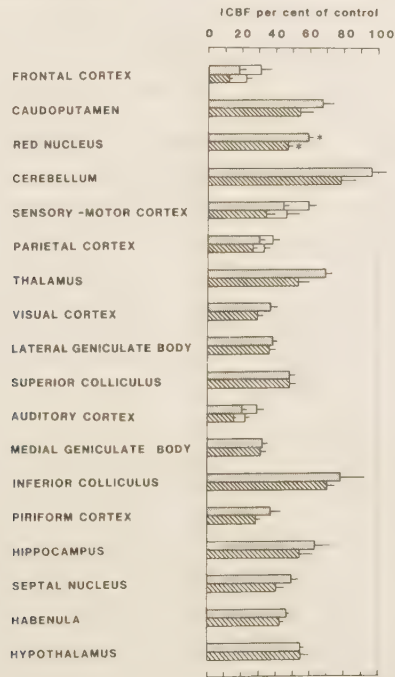


Fig. 9

Local CBF in per cent of control following 15 min of complete compression ischemia and recirculation of 60 min (shaded columns) and 90 min (striped columns) duration. In some structures relative flow rates have also been related to the unanesthetized control animals of Dahlgren et al. (1981) (unshaded columns). The difference between mean flow rates at 60 and 90 min, respectively, was statistically significant in the red nucleus only ($p < 0.05$). Values are means $\pm$ SEM.

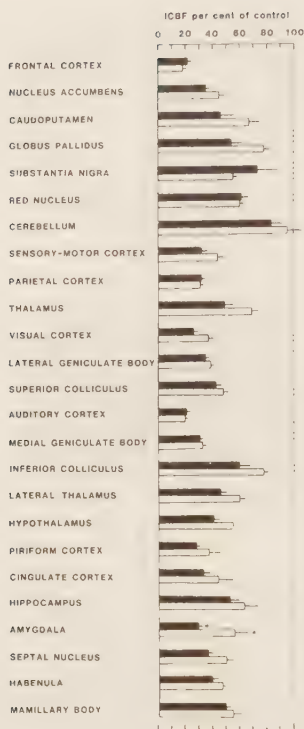


Fig. 10

Local CBF in per cent of control after 60 min of recirculation following 15 min of complete compression ischemia (unshaded columns) and 15 min of four-vessel-occlusion ischemia (shaded columns) respectively.

Fig. 10 compares the percentage reduction of l-CBF' after 60 min of compression and four-vessel-occlusion ischemia, respectively. In the majority of structures analyzed, the per cent reduction in flow rates was larger following four-vessel-occlusion than compression ischemia. However, results obtained after four-vessel-occlusion were similar in the sense that they confirmed the finding of marked heterogeneities in reflow (see cerebral cortices versus cerebellum, substantia nigra, and inferior colliculus).

DISCUSSION

When discussing the present results we will consider in turn the circulatory events in the immediate post-ischemic period and those characterizing the later stages of the recirculation period. Before doing so, we wish to emphasize that, in some structures, the post-ischemic decrease must have been exaggerated by the anesthetic procedure. Thus, in auditory and frontal cortex (see also parietal cortex) CBF was considerably curtailed by a very shortlasting increase in CSF pressure. These structures are the ones in which CBF is clearly raised above control by the anesthetic procedure (Dahlgren et al. 1981). It seems likely that the increase in CBF reflects the stress of anesthesia and immobilization, which is blunted by even very brief ischemia. At any rate, the results suggest that for these structures the true decrease in CBF following 15 min of ischemia should be assessed by comparisons with CBF values obtained in awake, unanesthetized animals.

1. No-reflow versus reactive hyperemia

As stated in the introduction, previous workers utilizing methods for direct measurements of CBF have concluded that, provided an adequate postischemic perfusion pressure can be instituted, post-ischemic CBF is characterized by an initial hyperemia and by a secondary "delayed" hypoperfusion. However, it has been difficult to exclude that a true no-reflow occurs since most studies have yielded overall CBF values; i.e. the methods have not allowed quantification of flow rates in structures prone to develop perfusion defects upon vascular perfusion with carbon black. The problem is highlighted by the results reported by Kofke et al. (1979) who found that in monkeys subjected to 16 min of ischemia, recirculation was followed by an increase of "regional" but not of "total" CBF, implying that CBF of some regions must have decreased. In this respect, the local CBF values reported by Hallenbeck (Hallenbeck and Bradley 1977, Hallenbeck and Furlow 1979) or by Ginsberg et al. (1978) give no hints since their measurements were confined to long recirculation periods (30 - 90 min). It would seem, therefore, that evidence against a primary no-reflow rests on the data reported by Marshall et al. (1975), by Levy et al. (1979), and by Pulsinelli et al. (1982 a). However, since Levy et al. (1979) induced incomplete ischemia (flow rates during

ischemia were $0.03 - 0.32 \text{ ml.g}^{-1}.\text{min}^{-1}$) and since Pulsinelli's model spares brain stem structures, their results may not be applicable to situations for which a no-reflow phenomenon has been claimed. It should be noted, though, that Levy et al. (1979) found patchy reperfusion immediately after release of the carotid clamp.

The present results unequivocally demonstrate that a true no-reflow phenomenon occurred following 15 min of ischemia in spite of the fact that a normal perfusion pressure was quickly restored at the termination of the ischemia. With the compression model, perfusion defects were observed in spite of the hemodilution caused by the seeping of artificial CSF into the general circulation. (As will be described in a forthcoming publication, the hematocrit in the pre-ischemic period, after 15 min of ischemia, and after 60 min of recirculation, was 50 ± 1 , 33 ± 1 , and 38 ± 1 %, respectively). Characteristically, the perfusion defects were largely confined to the caudoputamen, thalamus, and hippocampus, but they extended from these sites to affect nucleus accumbens, globus pallidus, and amygdala as well, and wedge-shaped defects of low flow rates were seen to extend into the frontal, parietal, and sensorimotor cortex. We note that the perfusion defects are similar in distribution to those described by Cantu and Ames (1969). In confirmation of data reported by that group, we found clear evidence of perfusion defects after 10 min of ischemia. However, after 15 min of ischemia the perfusion defects were more pronounced than those reported by Fiscner et al. (1979). One characteristic finding, though, was the pronounced interanimal variation. Thus, in each of the structures mentioned flow rates were normal or even increased in some of the animals studied. We interpret the results to show that the potential for a no-reflow phenomenon is at hand following 10-15 min of complete ischemia. However, whether or not no-reflow occurs will depend on such factors as the magnitude of the postischemic perfusion pressure. Three findings should be emphasized. First, although it has been previously assumed that a no-reflow phenomenon should not accompany "bloodless" ischemia (Ginsberg et al. 1978), the present results demonstrate that it does. Second, since perfusion defects were observed following four-vessel-occlusion they are not peculiar to the CSF compression model. Third, perfusion impairments following 15 min compression ischemia did not persist since CBF subsequently increased in the affected areas (see below).

However, unequivocal residues of no-reflow areas were observed after after 90 min of recirculation following 30 min of compression ischemia. Thus, with extended periods of ischemia (as used by e.g. Ginsberg et al. 1978, Hallenbeck and Furlow 1979) the "delayed hypoperfusion" may not be a secondary event which follows upon an initial hyperemia, but seems to represent a lingering perfusion defect that is present already in the beginning of the recirculation period.

One of the principal findings of the present study was the pronounced variability in reflow not only between animals, but also between structures. Thus whereas some structures usually showed low flow rates after 5 min of reperfusion (e.g. frontal and auditory cortex and hippocampus) others showed relatively marked hyperemia (e.g. cerebellum, lateral and medial geniculate bodies, inferior and superior colliculi). Since the mean CBF values for parietal and sensorimotor cortex were not increased above control one finds that structures generally considered to be vulnerable (hippocampus, cerebral cortices, caudoputamen, and thalamus) showed little evidence of postischemic hyperemia. Although this relationship may be fortuitous one cannot exclude that it reflects the vulnerability of these structures.

In view of the present results, and those reported by others, we conclude that complete ischemia of whatever cause carries the potential of inducing a no-reflow phenomenon, notably because non-perfused vessels require a relatively high opening pressure. It seems likely that this phenomenon, by extending the period of ischemia of affected tissue, carries a risk. The findings emphasize the therapeutic advantage of the "vascular flush" induced by full restoration (or an increase) of the postischemic perfusion pressure (Hossmann 1977).

2. Delayed hypoperfusion

The existence of a secondary hypoperfusion was established by workers using methods for measuring overall or regional ("cortical") CBF (Hossmann et al. 1973, 1976, Snyder et al. 1975, Nordström and Rehncrona 1977, Steen et al. 1978, Miller et al. 1980, Jackson et al. 1981). In some of these studies, and those in which local CBF was assessed, the true magnitude of the postischemic CBF reduction may have been blunted by the use of barbiturates (see Nordström and

Rehncrona 1977, Steen et al. 1978). Special importance should therefore be directed to results obtained in unanesthetized animals by Levy et al. (1979), who showed that CBF fell to about 50 per cent of control in the period 0.5 - 4 hours postischemically. In the present context, though, we will pay particular attention to those studies in which local CBF was assessed. In some of these, the period of ischemia was so long that it appears likely that the reduction in CBF reflected a persisting no-reflow phenomenon (Hallenbeck and Bradley 1977, Hallenbeck and Furlow 1979). A shorter period (15 min) was employed by Ginsberg et al. (1978) who demonstrated a homogenous reduction in CBF to 30-35% of control after 90 min of recirculation. However, since the ischemia was incomplete (see lactic acid values reported by Welsh et al. 1978), and since barbiturate anesthesia was used, their results are not directly comparable to those reported presently. It is also difficult to compare our results to those reported by Pulsinelli et al. (1982 a) who used an ischemic model that preserves flow to the brain stem structures.

The present (compression) model induces ischemia of uniform severity throughout the brain. Furthermore, the duration of ischemia is short enough to allow extensive recovery of cerebral energy state (Ljunggren et al. 1974, see also Nordström et al. 1978). It is of interest, therefore, that a pronounced delayed hypoperfusion developed, and that this hypoperfusion was heterogenous. In general, the results are in good agreement with those reported by Levy et al. (1979) and by Pulsinelli et al. (1982 a). However, the results give novel information on several aspects. First, although a "weighted" mean of all local CBF values indicates that CBF was reduced by about 50%, the flow rates were markedly heterogenous. For example, flow rates of 30% of control, or lower, were observed in frontal, parietal, sensorimotor, auditory, temporal, and cingulate cortex, as well as in medial geniculate, and CBF values exceeding 55% of control occurred in cerebellum, globus pallidus, thalamus, red nucleus, substantia nigra, and inferior colliculus. Extreme examples were provided by frontal cortex (10-15% of control) and cerebellum (70-90% of control). We note, though, that these differences become less pronounced if one takes the effect of the anesthetic procedure on lCBF into account. Second, with few exceptions values obtained after 60 and 90 min of recirculation were similar, suggesting that progressive worsening of

the hypoperfusion did not occur. In the period 60 to 90 min, the structures showing a significant or suggestive decrease in CBF were mostly those in which a no-reflow phenomenon was observed after 5 min recirculation. Tentatively, in these structures the period of "hyperemia" was delayed. (After 60 min of recirculation following four-vessel-occlusion, with probably less extensive no-reflow, such a delay could be expected to be less pronounced. It is conspicuous (fig 10) that the differences in flow rates between the two models were most pronounced in structures prone to develop no-reflow phenomenon. However, the difference was significant in one of these structures only, the amygdala). Third, although cerebral cortical structures, which showed relatively low CBF values after 5 min of recirculation, had a pronounced hypoperfusion after 60 - 90 min, and although the cerebellum showed just the opposite pattern, there was no strict relationship between "immediate" and "delayed" flow rates. Thus, the hippocampus maintained CBF values exceeding 50% of control at 60 and 90 min of recirculation.

As stated in the introduction, the main objective of the present study was to explore whether or not vulnerable regions in the brain show special characteristics in their postischemic circulatory responses. One previous study was devoted to histopathological alterations following compression ischemia of maximally 15 min duration (Brierly et al. 1975). In that study, animals recirculated for 30 min showed a few instances of ischemic cell damage, localized to the cerebral cortex and hippocampus and, in one of six animals, in the caudoputamen. It is clear from the present results that the only obvious correlation between these findings, and those reported presently, is that the vulnerable regions either show absence of initial hyperemia, or an overt no-reflow phenomenon. However, it seems premature to exclude the possibility that the localization of cell damage correlates to the degree of secondary hypoperfusion. Thus, in view of the fact that final cell damage "matures" over hours and days (Ito et al. 1975, Pulsinelli et al. 1982 b) the ultimate conclusion on this matter must await further histopathological analyses after more prolonged periods of recirculation.

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II

Recirculation in the Brain following Incomplete
Ischemia in the Rat

by

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Abstract

The objective of this study was to characterize local cerebral blood flow in the recirculation period following incomplete forebrain ischemia. Specifically, we wished to explore if perfusion defects developed in the immediate recirculation period, to study how initial hyperemia and delayed hypoperfusion at the local level were related to the severity of the preceeding ischemia, and to find out whether reflow was influenced by the nutritional state of the animals. To that end, forebrain ischemia of 15 min duration was induced in fed and fasted ventilated rats under 70% N₂O. Local CBF was measured with an autoradiographic technique at the end of ischemia, as well as 5 and 60 min following the start of recirculation. Control experiments were performed to examine the influence of ischemia on cerebral metabolic state on fed and fasted animals.

The ischemia reduced CBF to excessively low values (<5% of control) in many forebrain structures, including the cerebral cortices, caudoputamen, and the hippocampus. In spite of this, perfusion defects failed to appear after 5 min of recirculation. Instead, moderate to marked hyperemia was present in all previously ischemic structures. After 60 min of recirculation, pronounced hypoperfusion developed. The magnitude of the initial hyperemia was poorly related to the severity of the preceeding ischemia, but the latter partly determined the degree of delayed hypoperfusion. Thus, no or only moderate hypoperfusion developed in structures whose flow rates during ischemia exceeded 30-40% of control. Fasted animals had a better preserved flow to many structures than fed animals, indicating that the detrimental effect of feeding (or glucose infusion) has a circulatory component.

INTRODUCTION

In a preceeding article (Kågström et al. 1982a, submitted for publication) we reported on experiments designed to characterize local cerebral blood flow (LCBF) in the recovery period following complete brain ischemia, as induced in lightly anesthetized, artificially ventilated rats. In that article, we reviewed recent results favouring the view that even relatively shortlasting ischemia is followed by a reduction in overall and regional CBF ("delayed hypoperfusion") which occurs even though restitution of cerebral perfusion pressure may lead to an initial increase in CBF. The results of our study confirmed previous data in showing that ischemia of 10-30 min duration, whether induced by an increase in intracranial CSF pressure or by four-vessel-occlusion combined with arterial hypotension, leads to such a delayed hypoperfusion. However, the autoradiographic techniques used made it possible to reveal the appearance of a true "no-reflow" phenomenon, i.e. an initial hindrance to recirculation in centrally located brain structures. Since such underperfused structures coexisted with hyperemic zones, complete cerebral ischemia seems to create complex circulatory disturbances which do not conform to the simple sequence ischemia - initial hyperemia - delayed hypoperfusion. Furthermore, the results suggested that when complete ischemia is of long duration (30 min), an initial hyperemia may not develop at all, and establishment of an "adequate" flow rate can, at least in some structures, require extended recirculation periods.

The objective of the present study was to provide information on recirculation following incomplete ischemia in rats maintained under similar anesthetic conditions. The model of forebrain ischemia used (Nordström et al. 1978) maintains some circulation to a variety of brainstem structures but gives only a trickle of flow to cortical structures, hippocampus, and caudoputamen (see also data reported by Pulsinelli et al. 1982a). Nonetheless, cortical blood flow rates seem sufficient to allow some continued substrate supply; hence, the outcome of the ischemia differs between fed and starved animals (Kehncrona et al. 1981, Kalimo et al. 1981). The objective of this study was, therefore, to provide answers to the following questions. (1) Is the no-reflow phenomenon characteristic of complete global ischemia or does it occur also under the conditions of incomplete

forebrain ischemia of the type employed here? (2) How is the appearance and/or the magnitude of initial hyperemia and delayed hypoperfusion, respectively, related to the severity of the preceding ischemia? (3) Is the delayed hypoperfusion similar in fed and in starved animals, or is the beneficial effect of food withdrawal associated with higher blood flow rates?

In attempting to provide an answer to the first of these questions, we studied reflow patterns after 5 min of recovery, following 15 min of ischemia. The second question was approached by relating the "initial" (5 min) and "delayed" (60 min) recovery CBF values in 24 different structures to the values measured during ischemia. Since blood flow rates during recovery may be influenced both by the severity of the preceding ischemia, and by local differences in vascular reactivity, the CBF values recorded after incomplete, forebrain ischemia were compared to those measured at 5 and 60 min, respectively, following complete, four-vessel-occlusion ischemia (a model which also involves clamping of vessels and arterial hypotension). The third question was studied by measurements of CBF after 60 min of recirculation, following 15 min of ischemia, in fed and in starved animals.

METHODS

Operative, sampling, and analytical techniques

All experiments were performed on male Wistar rats (350-400 g) of an S.P.F. strain (Møllegaard Avlslaboratorium, Copenhagen). Fed animals had free access to tap water and rat pellets until operation. In fasted animals food was withdrawn during the night before the experiment but the animals had free access to water. After induction of anesthesia with 3% halothane the rats were tracheotomized and artificially ventilated with a mixture of 0.7% halothane in 30% O₂ and 70% N₂O during the subsequent operation. The femoral arteries, femoral veins, and one brachial artery were cannulated for blood sampling, continuous blood pressure recording, infusions, and injections. Goldplated copper screws were inserted into the skull bone for EEG recordings. The animals were paralyzed with tubocurarine chloride (2 mg.kg⁻¹ i.v.) and given heparin (100 I.U. i.v.). After completion of

the surgical procedure the halothane supply was discontinued and ventilation was adjusted to give arterial oxygen (PaO_2) and carbon dioxide (PaCO_2) tensions of about 100 and 33–40 mm Hg, respectively. The animals were then allowed a steady state period of 30 min before induction of ischemia.

Arterial blood was sampled anaerobically in glass capillaries for blood gas determinations. PaO_2 , PaCO_2 and pH were measured with microelectrodes (Eschweiler and Co., Kiel, and Radiometer, Copenhagen), operated at 37°C, with due corrections for the body temperature. Analysis of whole blood concentrations of glucose, lactate, and pyruvate were performed after sampling of arterial blood directly into liquid nitrogen.

In animals selected for studies of cerebral cortical metabolites the brains were frozen in situ with the technique described by Pontén et al. (1973) and then chiselled out under intermittent irrigation with liquid nitrogen and stored at -80°C until extraction. A fronto-parietal part of cerebral cortex was then taken bilaterally, weighed and extracted at -22°C. All metabolites were analyzed with the specific, enzymatic, fluorometric techniques of Lowry and Passonneau (1972; for analytical conditions, see Folbergrová et al. 1972). Metabolites were expressed as $\mu\text{mol.g}^{-1}$ of wet tissue. The energy state of the tissue was expressed as the energy charge of the adenine nucleotide pool according to Atkinson (1968).

Statistical comparison was performed using Student's t-test, $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$.

Reversible, incomplete forebrain ischemia

Incomplete ischemia was induced by bilateral occlusion of the common carotid arteries by rubber-coated clamps. (Nordström et al. 1978). Simultaneously, the mean arterial blood pressure was decreased to 50 mm Hg. This was accomplished by the use of a servo-system with an automatically controlled syringe which extracted blood from one femoral artery and, when necessary, reinfused the previously extracted blood into a femoral vein. During the ischemic period, a fall in the intracranial temperature was avoided by placing a heating bulb at a predetermined distance from the animals head. We restored the circulation to the brain by removal of the clamps on the carotid arteries and normalized the blood pressure by reinfusion of blood (see fig. 1,

below). During the recirculation period excessive systemic acidosis was counteracted by repeated i.v. injections of small doses of 0.6 M sodium bicarbonate (1.0-1.5 ml per animal) and ventilation volume was adjusted to compensate for an increase in PaCO_2 .

Reversible complete four-vessel-occlusion ischemia

Four-vessel-occlusion ischemia was induced with the method described by Kågström et al. (1982a). In summary, the common carotid and vertebral arteries were compressed bilaterally by a thread drawn through the paravertebral muscles and tied in the neck. To compress collaterals a cotton band was tied hard around the neck above the ligature. During ischemia mean blood pressure was lowered to about 50 mm Hg by i.v. infusion of Arfonad[®] (Tri-metaphan-camphor-sulphonate) and arterial bleeding. To restore the circulation to the brain we released the ties and normalized the blood pressure with blood infusion. Changes in intracranial temperature and in plasma acid-base state was avoided as described under the previous heading.

Measurement of local CBF (lCBF)

Local CBF was measured with the technique described by Landau et al. (1955) as modified by Sakurada et al. (1978). Details of procedure have been given in previous communications (Abdul-Ranman et al. 1979, Ingvar et al. 1981). In summary, 30-50 μCi of ^{14}C -iodoantipyrine, dispensed in 0.4 ml of Krebs-Hensleit solution, was infused i.v. at a constant rate for 30 sec (45 sec if a reduced lCBF was expected). Blood samples were obtained from the brachial artery catheter before and every third sec (5th sec) during the infusion. At 30 sec (45 sec) the animal was decapitated. The brain was then removed from the skull (within 2 min) and immediately immersed in isopentane chilled to -55 to -65°C. The ^{14}C activity in brain tissue (per unit mass) was evaluated by autoradiography. Slices of brain (20 μm) were cut in a Leitz sledge microtome at -20°C and applied to an X-ray film which was then exposed for one week. A series of calibrated ^{14}C -standards was applied to each film together with the brain slices. The film was then evaluated manually with a densitometer (McBeth, 1 mm aperture).

Local CBF was calculated as described by Sakurada et al.

(1978). With the sampling modification used it was not possible to determine lCBF accurately at all flow rates. In some structures the ^{14}C activity became so high that one reached the steep slope of the (logarithmic) curve which is obtained by plotting the ^{14}C activity in the brain tissue against the corresponding calculated CBF values. We chose to consider values inaccurate when a 2% error in the determination of the ^{14}C activity corresponded to a 10% variation in lCBF, as evaluated in each individual animal. If activities were higher, lCBF was assigned a value determined at that "break point". This means that lCBF values were underestimated in some structures, as indicated in fig. 5 and fig. 7 (see results).

Experimental groups

Local CBF at the end of 15 min of incomplete ischemia was evaluated in a group of 6 fed animals. Since very low CBF values were encountered in the majority of structures examined, the sampling procedure was slightly modified and 20 μCi of ^{14}C -iodoantipyrine was infused at a constant rate for 60 sec.

lCBF during recovery following incomplete ischemia was evaluated in 3 groups, each comprising 6 animals. Recirculation times was 5 min in one group of fed animals, and 60 min in 2 groups, one with fed and one with fasted animals.

Results of lCBF studies following four-vessel-occlusion ischemia were obtained from the previous communication (Kägström et al. 1982a).

Control values for lCBF were obtained from animals kept artificially ventilated for periods corresponding to the duration of the experiments. Anesthesia, as well as operative and sampling techniques, were the same as in the experimental groups. The control groups included 7 fed animals and 5 fasted animals.

The objective of the measurements of labile cerebral cortical metabolites was to assess the degree of metabolic derangement associated with the reduction in CBF, and to compare changes in fed and starved animals (see Discussion). Since we also wished to study recovery events, an initial series comprised four groups of fed rats which were exposed to 15 min of incomplete ischemia, with recirculation times of 0, 5, 15, and 30 min, respectively. However, due to an error in tissue extraction, the results varied unduly. Therefore, only

the blood changes from these groups are reported here. In order to allow a comparison of changes in tissue metabolites in fed and fasted animals two additional groups were prepared, in which tissue freezing occurred after 15 min of ischemia. The results were compared to those obtained after an identical period of complete, four-vessel-occlusion ischemia in fed animals.

Control values for concentrations of phosphocreatin and adenine nucleotides as well as glucose, lactate, and pyruvate in cerebral cortex, were obtained from two control animals treated in a similar way as the lCBF control animals. Since the values agreed well with the results obtained in 4 control animals in a concurrent study, the values were pooled with these to give a group of 6 animals.

R E S U L T S

The present model of forebrain ischemia has been used in previous studies from the laboratory (Nordström et al. 1978, Rehncrona et al. 1979, 1981) and a similar method has been employed in the cat (Welsh et al. 1978). Furthermore, the model designed by Pulsinelli seems to give similar CBF values in many brain structures (Pulsinelli et al. 1982a). In spite of this we deemed it desirable to further characterize the model in terms of changes in physiological and biochemical variables, especially since we are presently adopting it for studies of long-term survival. To that end separate series were designed to provide data on blood acid-base changes, and on biochemical events in cerebral cortical tissue (see methods).

1. Physiological and chemical variables

Following clamping of the common carotid arteries, and lowering of the mean arterial blood pressure to 50 mm Hg, EEG activity ceased within 15 to 25 sec. In none of the animals in the present series did any EEG activity return during the 15 min period of ischemia.

Fig. 1 shows the time course of changes in arterial blood pressure. Values close to those reported for the 5 min recovery (see below) were obtained within the first 2 min, following removal of the carotid clamps, and the reinfusion of blood. During the first 5 min,

peak mean arterial blood pressures varied among the animals between 120 and 150 mm Hg.

In the separate series devoted to measurements of blood variables, glucose concentrations were measured at four times in fed and fasted animals. Fig.2 shows marked differences between the two series. Thus, although preischemic blood glucose concentrations differed by only $3 \mu\text{mol.g}^{-1}$, fed animals showed a pronounced rise in glucose values during ischemia while those of fasted animals fell.

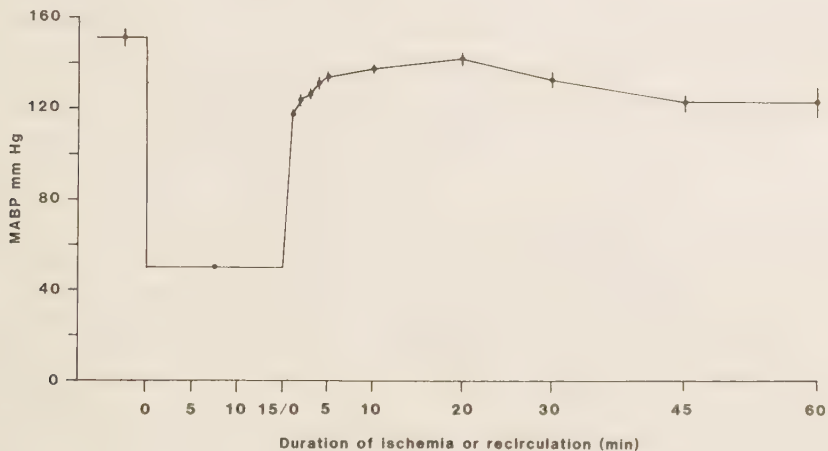


Fig. 1

Mean arterial blood pressure before, during, and following 15 min incomplete forebrain ischemia.

In spite of the differences between the groups in blood glucose concentrations there were no clear differences in plasma acid-base changes, or in lactate and pyruvate concentrations. Fig. 3 demonstrates, therefore, only the values obtained in fed animals. In spite of an appreciable increase in blood lactate (and pyruvate) concentrations during ischemia, plasma pH did not fall significantly. In all probability, this was due to a reduction in PCO_2 (which occurred at constant ventilation). Five min after recirculation PCO_2 increased with maintained lactic acidosis. As a result, plasma pH fell appreciably. However, from 15 min onwards pH could be maintained close to control values by i.v. bicarbonate infusion (see methods). As fig. 3 shows, lactate and pyruvate concentrations remained moderately increased throughout the recirculation period.

Table 1 shows the values for cerebral cortical metabolites, as measured at the end of 15 min of ischemia. In both fed and fasted animals, subjected to incomplete ischemia, the deterioration of cerebral energy state was similar to that obtained in complete four-vessel-occlusion ischemia. Thus, PCr and ATP concentrations were close to zero and massive accumulation of ADP and AMP occurred. In both groups, glycogen was depleted and pyruvate concentrations reduced to low values. However, the groups differed with respect to glucose and lactate concentrations. Thus, fed animals maintained significantly higher glucose concentrations and accumulated more lactate. Two findings should be emphasized. First, the amount of lactate accumulated in fasted animals was similar to that observed during complete

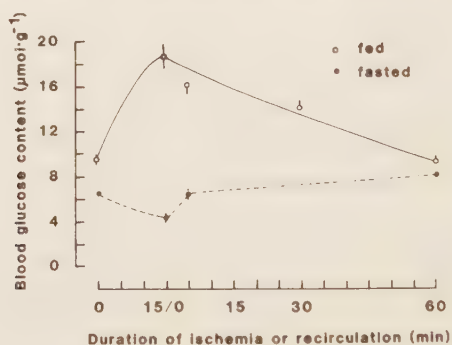


Fig. 2

Blood glucose concentration before, during, and following 15 min of incomplete ischemia in fed and fasted animals. Values are means $\pm$ SEM.

ischemia in fed animals. Second, two fed animals had lactate concentrations below 20 $\mu\text{mol.g}^{-1}$ (13.9 and 15.8 $\mu\text{mol.g}^{-1}$, respectively) in spite of markedly low ATP concentrations (0.10 and 0.05 $\mu\text{mol.g}^{-1}$, respectively). Probably, such values reflect virtually complete cessation of blood flow (see discussion).

2. Cerebral blood flow during and following incomplete ischemia

Local CBF was measured at the end of a 15 min period of ischemia as well as following 5 min of recirculation in fed animals, and following 60 min of recirculation in both fed and fasted animals.

Table 1. Preischemic blood glucose and cerebral cortical metabolites at the end of 15 min ischemia.

Experimental groups	glucose/bl	PCr	ATP	ADP	AMP	E.C.	glyco- gen	glu- cose	lac- tate	pyru- vate	La/Py	n
Controls, fed animals	8.1 +2.1	4.59 +0.09	2.51 +0.05	0.279 +0.008	0.064 +0.014	0.929 +0.005	(2.50)* +0.43	3.06 +0.34	1.69 +0.008	0.127 +0.008	12.8 +2.0	6
Four-vessel-occlusion 15', fed animals	13.2 +1.4	0.18 +0.05	0.08 +0.02	0.662 +0.046	1.80 +0.16	+0.160 +0.005	0.18 +0.03	+0.38 +0.05	15.50 +0.70	0.030 +0.008	795 +248	6
Incomplete ischemia 15', fed animals	8.9 +0.8	0.06 +0.02	0.11 +0.02	0.586 +0.064	1.72 +0.06	0.167 +0.020	0.09 +0.01	0.60 +0.13	19.5 +1.6	0.021 +0.002	1000 +142	6
Incomplete ischemia 15', fasted animals	6.6 +0.2	0.08 +0.02	0.08 +0.02	0.628 0.065	1.63 +0.05	0.168 +0.013	0.06 +0.01	0.22 +0.04	14.2 +0.8	0.021 +0.004	750 +70	6

The concentrations are expressed in $\mu\text{mol} \cdot \text{g}^{-1}$ blood and tissue, respectively. Values are means $\pm$ SEM.

* Only two animals were analysed for concentrations of glycogen in cerebral cortex, but this a normal value in this strain (E.G. Folbergrová et al. 1981, Rehnrcrona et al. 1981).

Physiological parameters for these series are given in table 2. Acid-base changes were similar to those given above (see fig. 3). In the groups where LCBF was measured during recirculation all animals but one (60 min recovery, fed animal, blood pressure 90 mm Hg) had mean arterial blood pressures of 120 mm Hg, or higher, prior to LCBF measurement.

Table 3 gives the LCBF values for 24 different brain structures in the six groups studied. In none of the structures analysed did the CBF values for fed and fasted control groups differ (the range of values in fasted animals, in per cent of fed control values, were between 90 and 118 per cent). Values obtained during and following ischemia will be commented upon under separate headings.

a. Local CBF during ischemia. Predictably, CBF during ischemia varied considerably between different structures. If one considers mean values, as obtained by averaging the densitometric readings for each structure as well as the values for all animals, three groups can be distinguished. First, values below 5% of control were obtained in all cerebral cortical structures (frontal, sensorimotor, parietal, piriform, cingulate, auditory, and visual), as well as in caudoputamen, amygdala, and hippocampus. Second, values below 15% of control were obtained in nucleus accumbens, septal nucleus, globus pallidus, thalamus, and the geniculate bodies. Third, higher values (>15% of control) were seen in hypothalamus, habenula, the colliculi, and, particularly, in the red nucleus, substantia nigra,

and cerebellum.

The values depicted in table 3 give no information on inter-animal variations, or on variations in CBF within structures. Such variations are illustrated in fig. 4, which lists the structures in a way that facilitates inspection of interstructural differences in CBF.

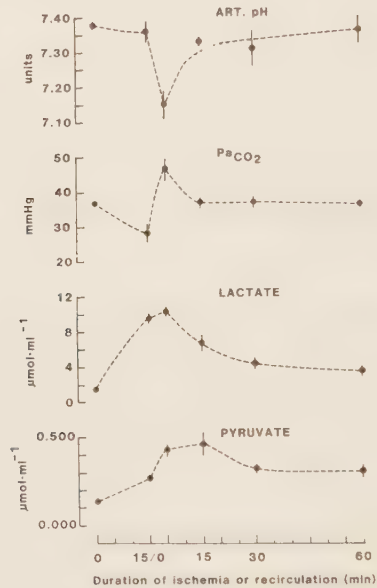


Fig. 3

Plasma acid-base and blood lactate as well as pyruvate concentrations before, during, and following 15 min of incomplete ischemia in fed animals. Values are means $\pm$ SEM.

This order of sequence (low $\rightarrow$ high CBF) will be used also in the following figures. The diagram makes it possible to identify a group of structures in which all readings showed CBF values consistently below 5% of control (frontal, parietal, sensorimotor, piriform, auditory, and visual cortex), and another one in which the CBF in at least 5/6 animals was below 15% of control (nucleus accumbens, caudoputamen, septal nucleus, cingulate cortex, amygdala, and hippocampus). We note that structures considered vulnerable in models of forebrain ischemia (see Pulsinelli et al. 1982 b) belong to these groups. More variable CBF values (but still below 40% of control in 5/6 animals)

Table 2. Physiological parameters in control animals and in animals subjected to 15 min incomplete ischemia.

Experimental groups		Temp. °C	MABP mm Hg	PaO ₂ mm Hg	PaCO ₂ mm Hg	pH	n
Fed control animals		37.1 ±0.1	152 ±2	100 ±5	36.8 ±0.8	7.41 ±0.01	7
Fasted control animals		37.3 ±0.2	144 ±2	100 ±3	35.7 ±0.5	7.40 ±0.01	5
15 min incompl. ischemia	preisch.	36.9 ±0.2	151 ±4	106 ±4	37.2 ±1.4	7.39 ±0.02	6
	15' isch.	37.3 ±0.1	50 ±0	115 ±6	29.3 ±3.1	7.28 ±0.02	6
15 min incompl. ischemia, 5 min recirc.	preisch.	37.0 ±0.2	158 ±3	104 ±4	39.1 ±1.1	7.36 ±0.02	6
	5' recirc.	37.1 ±0.1	143 ±3	119 ±1.3	49.3 ±2.3	7.11 ±0.06	6
15 min incompl. ischemia, 60 min recirc. Fed rats	preisch.	37.1 ±0.1	147 ±3	106 ±4	37.6 ±0.7	7.40 ±0.02	6
	15' recirc.	37.3 ±0.2	135 ±2	123 ±8	35.9 ±1.7	7.31 ±0.02	6
	60' recirc.	37.3 ±0.1	123 ±7	105 ±7	35.9 ±0.9	7.36 ±0.04	6
15 min incompl. ischemia, 60 min recirc. Fasted rats	preisch.	37.0 ±0.2	147 ±3	108 ±4	34.4 ±1.4	7.40 ±0.02	5
	15' recirc.	37.7 ±0.4	135 ±4	117 ±12	34.0 ±1.5	7.44 ±0.01	5
	60' recirc.	37.1 ±0.2	120 ±0	117 ±2	36.6 ±2.1	7.40 ±0.04	5

were recorded in globus pallidus, thalamus, hypothalamus, habenula, the geniculate bodies, and superior colliculus). The highest flow rates were obtained in the cerebellum, red nucleus, substantia nigra, and inferior colliculus. Clearly, this interstructural variation in flow rates must reflect continued blood supply by the vertebral arterial system, and by collaterals.

b. Local CBF after 5 min of recirculation. As table 3 shows (see above), very high CBF values were recorded in the "immediate" recirculation period. In order to illustrate interanimal variations, mean and individual values have been given in per cent of control in fig. 5. The illustration of average values for all structures in any given animal seems justified in view of the fact that intrastructural variations were small, or absent. However, the figures given underestimate flow rates in some structures, since in these the lCBF values exceeded the respective "breakpoints" (see methods). The number of animals in which this occurred is given in the figure. The results demonstrate that hyperemia was present in all structures after 5 min of recirculation, and that perfusion defects were not observed. However, the relative increase in flow varied between structures. The lowest values (mean CBF <200% of control) were observed in frontal and auditory cortex, red nucleus, and cerebellum. In the last two structures, lCBF was only moderately reduced during ischemia but, in the

first two, this cannot have contributed to the fact that such moderate hyperemia occurred. Marked increases (>350% of control) were observed in septal nucleus, globus pallidus, thalamus, substantia nigra, superior colliculus, and hippocampus. In the remaining structures, flow rates were intermediate. In some of these, though, the actual flow rates must have been higher than indicated (see figures for number of underestimated values).

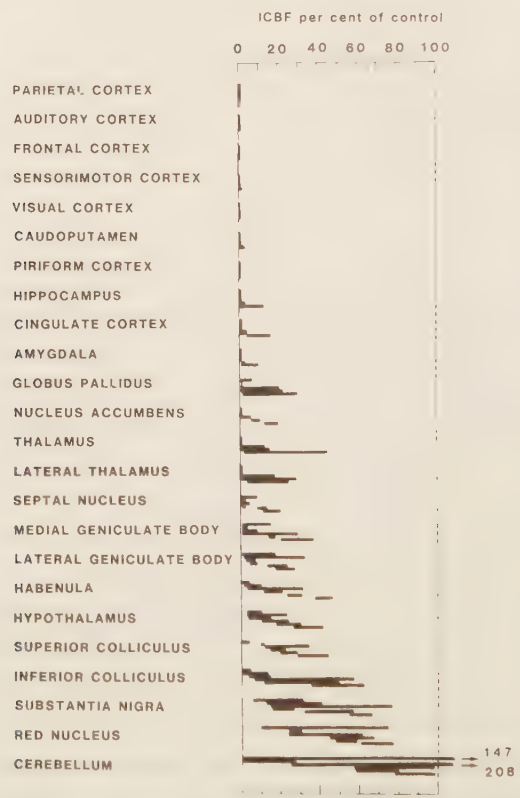


Fig. 4

Individual variations of local CBF during incomplete ischemia. The 24 cerebral structures examined are arranged in order of severity of ischemia, based on mean flow values in per cent of control calculated from the mean values depicted in table 3. The upper and lower ends of each staple represent the flow values in per cent of control corresponding to the highest and lowest densitometric reading, respectively, in each animal. If a staple touches the baseline, the lowest flow value is zero.

Table 3. Local cerebral blood flow in control animals and during, as well as following, 15 min incomplete forebrain ischemia.

Structure	Fed controls (n=7)	Fasted controls (n=5)	15' isch. Fed rats recirc. 0 (n=6)	15' isch. Fed rats recirc. 5' (n=6)	15' isch. Fed rats recirc. 60' (n=6)	15' isch. Fasted rats recirc. 60' (n=6)
Frontal cortex	2.03 $\pm$ 0.08	2.05 $\pm$ 0.15	0.01	2.96 $\pm$ 0.31	0.26 $\pm$ 0.09	0.45 $\pm$ 0.06
Nucl. accumbens	1.50 $\pm$ 0.10	1.60 $\pm$ 0.04	0.08	3.59 $\pm$ 0.22	0.42 $\pm$ 0.08	0.57 $\pm$ 0.04
Caudoputamen	1.41 $\pm$ 0.09	1.56 $\pm$ 0.07	0.02	3.30 $\pm$ 0.55	0.38 $\pm$ 0.07	0.63 $\pm$ 0.06
Sensorimotor cortex	1.98 $\pm$ 0.10	1.82 $\pm$ 0.17	0.01	5.73 $\pm$ 0.43	0.40 $\pm$ 0.08	0.72 $\pm$ 0.11
Septal nucleus	0.85 $\pm$ 0.06	0.85 $\pm$ 0.06	0.07	3.06 $\pm$ 0.28	0.28 $\pm$ 0.07	0.39 $\pm$ 0.05
Globus pallidus	0.66 $\pm$ 0.07	0.69 $\pm$ 0.05	0.03	2.75 $\pm$ 0.18	0.28 $\pm$ 0.03	0.32 $\pm$ 0.03
Thalamus	1.63 $\pm$ 0.09	1.68 $\pm$ 0.09	0.10	6.02 $\pm$ 0.39	0.41 $\pm$ 0.11	0.57 $\pm$ 0.06
Hypothalamus	1.05 $\pm$ 0.08	1.07 $\pm$ 0.09	0.17	2.40 $\pm$ 0.33	0.51 $\pm$ 0.13	0.71 $\pm$ 0.10
Habenula	1.64 $\pm$ 0.12	1.67 $\pm$ 0.07	0.29	3.96 $\pm$ 0.59	0.76 $\pm$ 0.13	1.03 $\pm$ 0.12
Lateral thalamus	1.53 $\pm$ 0.09	1.65 $\pm$ 0.07	0.11	6.18 $\pm$ 0.26	0.44 $\pm$ 0.08	0.64 $\pm$ 0.06
Parietal cortex	1.89 $\pm$ 0.09	2.29 $\pm$ 0.23	0.01	4.71 $\pm$ 0.60	0.38 $\pm$ 0.16	0.59 $\pm$ 0.11
Piriform cortex	1.12 $\pm$ 0.10	1.27 $\pm$ 0.11	0.01	2.32 $\pm$ 0.16	0.35 $\pm$ 0.13	0.41 $\pm$ 0.03
Cingulate cortex	1.33 $\pm$ 0.12	1.27 $\pm$ 0.10	0.02	4.14 $\pm$ 0.45	0.33 $\pm$ 0.08	0.52 $\pm$ 0.06
Amygdala	1.06 $\pm$ 0.09	1.20 $\pm$ 0.07	0.02	3.32 $\pm$ 0.24	0.25 $\pm$ 0.06	0.37 $\pm$ 0.04
Hippocampus	0.87 $\pm$ 0.07	0.98 $\pm$ 0.12	0.01	3.23 $\pm$ 0.41	0.28 $\pm$ 0.06	0.34 $\pm$ 0.02
Lateral geniculate body	1.40 $\pm$ 0.11	1.42 $\pm$ 0.06	0.18	4.79 $\pm$ 0.65	0.39 $\pm$ 0.09	0.55 $\pm$ 0.06
Red nucleus	1.45 $\pm$ 0.10	1.49 $\pm$ 0.06	0.66	2.45 $\pm$ 0.55	1.01 $\pm$ 0.26	1.22 $\pm$ 0.07
Substantia nigra	1.01 $\pm$ 0.07	1.03 $\pm$ 0.07	0.37	3.96 $\pm$ 0.51	0.90 $\pm$ 0.32	0.88 $\pm$ 0.05
Medial geniculate body	1.82 $\pm$ 0.09	2.09 $\pm$ 0.11	0.21	6.07 $\pm$ 0.58	0.59 $\pm$ 0.11	0.80 $\pm$ 0.10
Superior colliculus	1.45 $\pm$ 0.11	1.32 $\pm$ 0.08	0.30	5.66 $\pm$ 0.56	0.77 $\pm$ 0.16	0.98 $\pm$ 0.07
Auditory cortex	3.12 $\pm$ 0.15	3.47 $\pm$ 0.28	0.01	5.27 $\pm$ 0.61	0.51 $\pm$ 0.18	0.79 $\pm$ 0.13
Inferior colliculus	1.99 $\pm$ 0.09	1.79 $\pm$ 0.17	0.39	6.18 $\pm$ 0.49	1.74 $\pm$ 0.56	1.73 $\pm$ 0.17
Visual cortex	1.52 $\pm$ 0.08	1.49 $\pm$ 0.09	0.01	3.45 $\pm$ 0.40	0.36 $\pm$ 0.13	0.58 $\pm$ 0.07
Cerebellum	0.82 $\pm$ 0.06	0.81 $\pm$ 0.07	0.51	1.42 $\pm$ 0.56	0.78 $\pm$ 0.33	1.06 $\pm$ 0.09

Local CBF is expressed in $\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. Values are means $\pm$ SEM. In animals examined at the end of 15 min ischemia there was pronounced intrastructural heterogeneity and only mean values are given.

c. Local CBF after 60 min of recirculation. The pronounced interstructural variation in CBF after 60 min of recovery (Table 3) is evident by the results of fig. 6 which also gives a comparison between fed and fasted animals. In fed animals, a number of structures had mean flow rates below 1/3 of control. These included all cerebral cortical areas, caudoputamen, nucleus accumbens, thalamus, the geniculate bodies and the hippocampus. The reductions in frontal, auditory, sensorimotor, and parietal cortex were very marked (13, 16, 20, and 20 % of control, respectively). However, these are structures in which increases in CBF due to the anesthetic procedure are pronounced, and when the comparison is made to unanesthetized animals, the percentage reduction is less marked (25-30 % of control, cf Kågström et al.

1982a). In a number of structures, flow rates exceeded 40 % of control (globus pallidus, hypothalamus, habenula, substantia nigra, inferior colliculus, and cerebellum). In at least three (globus pallidus, hypothalamus, and habenula), the high perfusion rates occurred in spite of the fact that ischemic flow rates were reduced below 20 % of control (see above).

Significant differences between fed and fasted animals were observed in 10 of the 24 structures. However, since the mean CBF values were higher in fasted than in fed animals in all but two structures (whose recovery flow rates were close to control) it seems that fasting did enhance recovery flow rates, the effect being most pronounced in structures whose flow rates during ischemia were low.

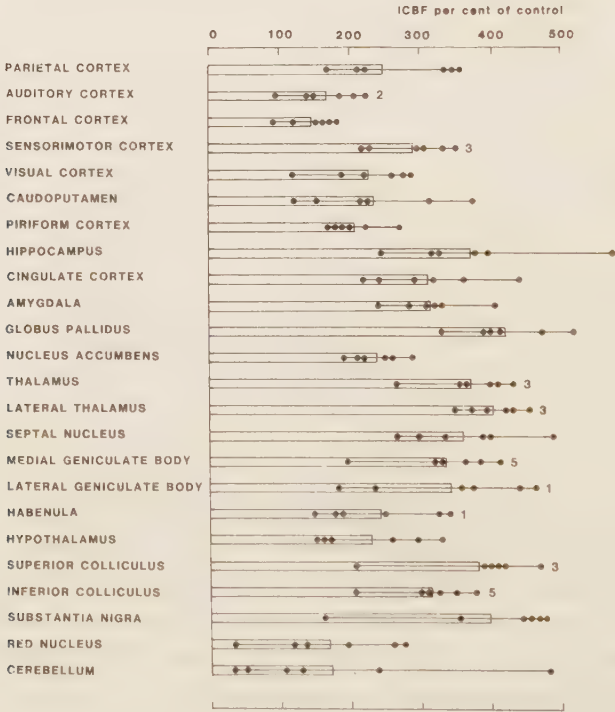


Fig. 5

Mean and individual ICBF values in per cent of control in 24 brain structures after 5 min of recirculation following 15 min of incomplete ischemia. The structures are arranged in order of severity of ischemia from left to right. A figure to the right of a staple indicates the number of experiments in the group in which the CBF value is underestimated (see text).

DISCUSSION

Before we discuss the present results, and their relevance for the three questions posed in the introduction, it seems justified to consider the severity of the ischemia induced by the present methods. Clearly, although the CBF technique used is well suited to provide answers to the main problems studied in the present context it does not accurately resolve differences in CBF in severe ischemia, e.g. when flow rates fall below 10% of control. A further difficulty arises from the fact that CBF during 15 min of incomplete ischemia is estimated from a single value measured at the end of this period. In fact, many structures had such low calculated flow rates that it becomes a matter of speculation whether the ischemia was incomplete or complete. It is of some importance, therefore, that deductions regarding CBF can be made from results on the amount of lactate accumulated.

With the present techniques for inducing and maintaining anesthesia, tissue glycogen concentration is around $2.5 \mu\text{mol.g}^{-1}$, the lactate concentration around $1.5 \mu\text{mol.g}^{-1}$, and the tissue to blood glucose concentration ratio close to 0.4. Since the animals exposed to complete ischemia had a blood glucose concentration of $13 \mu\text{mol.g}^{-1}$, one would expect a tissue glucose concentration of $5 \mu\text{mol.g}^{-1}$. Hence, complete interruption of cerebral circulation would be expected to give a final lactate concentration of $16\text{--}17 \mu\text{mol.g}^{-1}$. Clearly, the value measured ($15.5 \mu\text{mol.g}^{-1}$) does not exceed that value, demonstrating that the ischemia must have been complete (see Kågström et al. 1982a, and also Ljunggren et al. 1974). In the fed animals exposed to incomplete ischemia the blood glucose concentration measured ($9 \mu\text{mol.g}^{-1}$) would correspond to a tissue glucose concentration of about $3.5 \mu\text{mol.g}^{-1}$. In these animals, therefore, complete ischemia would be expected to give a final lactate concentration of about $14 \mu\text{mol.g}^{-1}$. In starved animals, with a blood glucose concentration of $6.5 \mu\text{mol.g}^{-1}$ (fig. 2), the corresponding value for lactate would be $11.5 \mu\text{mol.g}^{-1}$.

From these calculations, we can deduce the following. First, since lactate concentrations in fed animals exposed to incomplete ischemia exceeded $15 \mu\text{mol.g}^{-1}$ in all rats but one, some flow must have remained in these animals. In four of six animals, lactate concentrations exceeded $20 \mu\text{mol.g}^{-1}$, suggesting that $3\text{--}5 \mu\text{mol.g}^{-1}$ of glucose

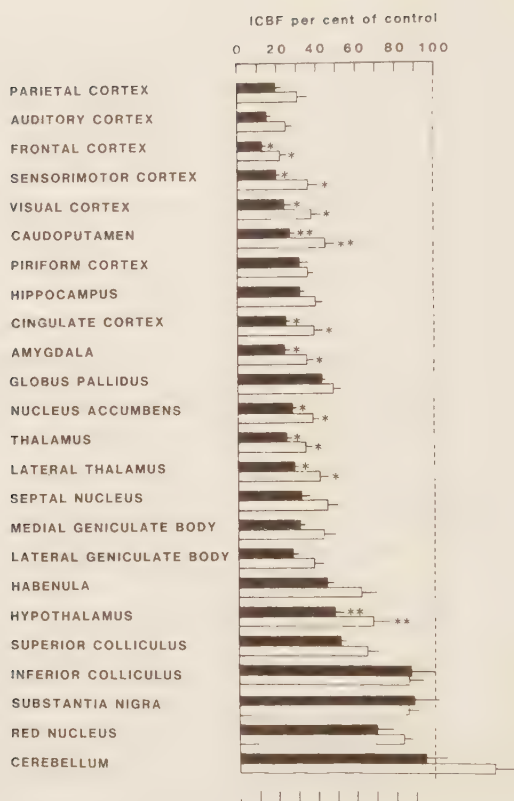


Fig. 6

Local CBF in per cent of control after 60 min of recirculation following 15 min of incomplete ischemia in fed (dark staples) and fasted (open staples) animals. Values are means $\pm$ SEM.

had been translocated from blood to tissue. If all glucose were extracted from blood, this would require flow rates of 1-2 % of control. Since this cannot probably be the case one must assume an average flow rate of around 5 % of control to account for the highest lactate concentration, and one close to zero to account for the lowest. Similar estimates are obtained if data for fasted animals are used (see table 1). Thus, only one lactate value was close to that expected in a situation of complete ischemia. In the remaining five, the lactate concentrations suggest that 1 - 3 $\mu\text{mol.g}^{-1}$ of glucose had entered the ischemic tissue. We conclude, therefore, that recorded

flow rates of less than 5 % of control correspond to a situation in which a trickle of blood flow persists, and that the low CBF values recorded in many structures represent reasonable estimates of the true, average perfusion rates.

With this information at hand, we can proceed to discuss the questions posed in the introduction.

1. The no-reflow phenomenon in complete versus incomplete ischemia.

The present results have clearly documented that failure of reperfusion following restoration of perfusion pressure does not occur in incomplete ischemia, even if ischemic flow rates fall towards zero. As described in the preceeding article (Kågström et al. 1982a), no-reflow areas tend to be localized to central, subcortical structures, especially to the caudoputamen. In the present study, during incomplete ischemia, CBF values in the caudoputamen were below 2 % of control in 4 of 6 animals; yet, all 5 min recovery values were markedly hyperemic. Possibly, the absence of perfusion defects following incomplete ischemia is related to the fact that severe ischemia affects only part of the brain. However, it seems even more likely that the no-reflow phenomenon is confined to situations with complete cessation of blood flow through capillary and precapillary vessels, possibly because blood during recirculation is "shunted" through vessels whose opening pressure is rapidly reached (Fischer and Ames 1972).

2. Influence of degree of ischemia on the magnitude of initial hyperemia and the severity of delayed hypoperfusion.

As stated in the introduction, one of the objectives of the present study was to explore how initial and delayed reflow CBF values relate to the severity of the preceeding ischemia. It must be expected that the appearance of an initial hyperemia requires that blood flow during the ischemia falls below a limiting value but, without further information, it cannot be predicted that a relationship exists between the severity of ischemia and the magnitude of postischemic hyperemia. Fig.5 (see above) shows that some structures with relatively well preserved flow during ischemia showed only moderate hyperemia during recovery, suggesting that, in these, ischemic flow rates were close to the "limiting" values. However, the results of fig.5 demonstrate that

no general relationship exists between the severity of ischemia and the magnitude of initial hyperemia. For example, two structures with relatively well preserved flow during ischemia (substantia nigra and inferior colliculus) had pronounced "reactive" hyperemia while frontal, parietal, temporal, and auditory cortex had flow rates of less than 250% of control during recovery in spite of the excessive reduction in CBF during ischemia. Finally, we recall that local differences

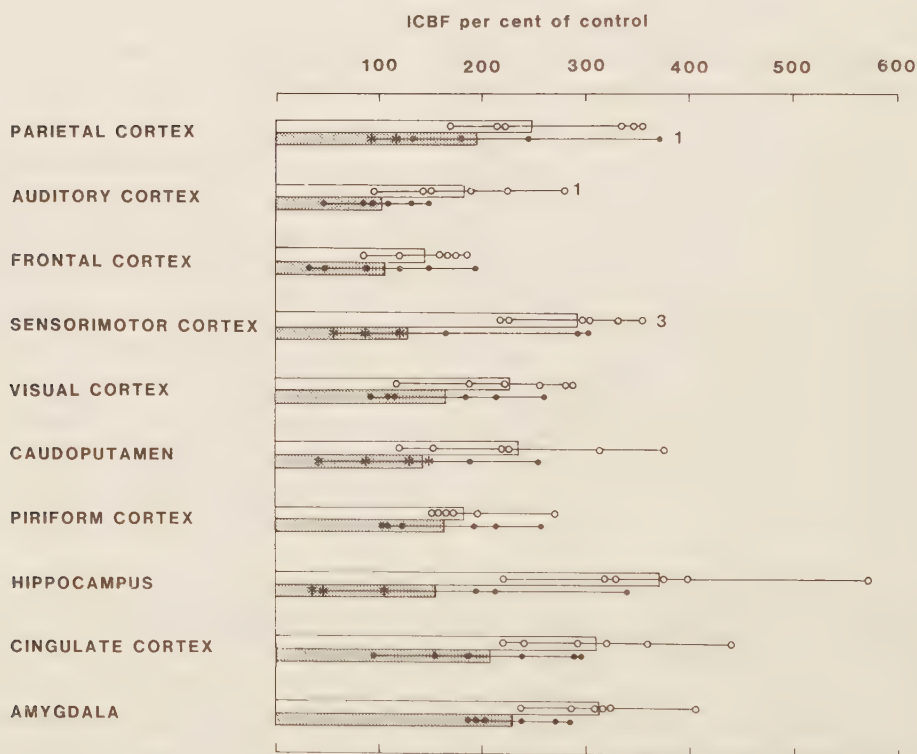


Fig. 7

Mean and individual ICBF values in per cent of control after 5 min of recirculation following 15 min of incomplete ischemia (open staples) and complete four-vessel-occlusion ischemia (dark staples). Only those structures are included in which ICBF during incomplete ischemia was less than 5% of control. * indicates the occurrence of no-reflow phenomenon in the structure. A figure to the right of a staple indicates the number of experiments in the group in which the CBF value is underestimated.

in initial reflow occur also when the ischemic insult is uniform, i.e. in complete ischemia (Kågström et al. 1982a).

It is of interest to compare the initial hyperemia following complete ischemia to that observed presently in those 10 structures whose blood flow rates were consistently below 5% of control during the ischemia (fig.7). One finds higher blood flow rates following incomplete ischemia in all these structures. However, the CBF values of fig.7 are influenced by the fact that many structures had no-reflow areas following complete ischemia. Thus, in perfused parts blood flow rates were closer to those measured following incomplete ischemia.

These observations point to another aspect of the results on initial flow rates which merits discussion. It is generally assumed that the initial hyperemia reflects vasoparalysis, possibly related to tissue acidosis. In this study measurements of cerebral metabolites were confined to structures with flow rates of less than 5% of control during ischemia. In regions with less severe ischemia there is a greater potential for lactate accumulation. If the tissue concentration of lactate at the end of ischemia is relevant for the degree of postischemic hyperemia, high flow rates could be expected in structures in which the ischemia is severe enough to stimulate glycolysis and induce lactate accumulation, yet sufficiently moderate to allow ample supply of substrate. Some support for such a relationship is afforded by the fact that following incomplete ischemia, the mean flow rates in structures that had been severely ischemic (<5% of control) averaged 250% of control, whereas the corresponding figures for structures with less severe ischemia (5-40% of control) were about 350% of control. However, this suggested difference is less evident if the comparison is made to unanesthetized control animals (cf Results). We must conclude that the data presented in this study do not permit any conclusion concerning a possible relation between the degree of ischemia and the magnitude of initial hyperemia. The problem will be explored further in a forthcoming publication (Kågström et al. 1982b, in preparation).

The present results make it more likely that some relationship exists between the severity of ischemia and the degree of secondary hypoperfusion. Thus, in fed animals all structures in which CBF during ischemia fell below 5% of control had recovery values less than 40% of control, while the eight structures that had recovery CBF

values of 50% of control, or higher, were those that suffered only moderate ischemia (habenula, hypothalamus, the colliculi, red nucleus, substantia nigra, and cerebellum). However, although these relationships are suggestive it must be recalled that interstructural differences in recovery CBF values exist also in complete ischemia, which leads to a uniform ischemic insult. For example, the results of fig.8 show that the four structures showing the highest flow rates following incomplete ischemia also had relatively high CBF values in the recovery period following complete ischemia, and that this type of

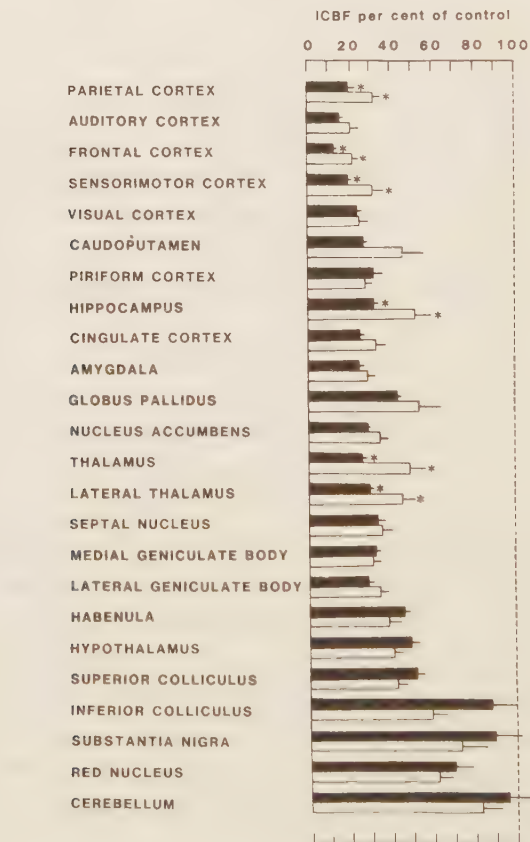


Fig. 8

Local CBF in per cent of control after 60 min of recirculation following 15 min of incomplete ischemia (open staples) and complete four-vessel-occlusion ischemia (dark staples). Values are means $\pm$ SEM.

ischemia leads to relatively pronounced differences in recovery CBF values for forebrain structures. We must conclude, therefore, that the phenomenon of delayed hypoperfusion is related both to the severity of the preceding ischemia, and to undefined vascular or metabolic characteristics of different brain structures.

3. Does the nutritional state influence the severity of delayed hypoperfusion

It is now clearly documented that feeding, or glucose infusion, adversely affects recovery following ischemia (Myers and Yamaguchi 1977, Myers 1979, Siemkowicz and Hansen 1978, Ginsberg et al. 1980, Welsh et al. 1980, Rehncrona et al. 1980, 1981, Kalimo et al. 1981). The results of one group indicate that this detrimental effect involves circulatory failure (Ginsberg et al. 1980, Welsh et al. 1980). However, in that study blood glucose concentrations were very high and the control group, in which glucose was not infused, had tissue lactate concentrations of close to $30 \mu\text{mol.g}^{-1}$.

In the present study, we chose to examine reflow in fed and fasted animals, making no attempts to raise tissue lactate levels to excessively high values. In spite of this, the results indicated that fasting is associated with higher local CBF values in animals allowed 60 min of recirculation following 15 min of incomplete forebrain ischemia. Clearly, the results give no hints to the underlying mechanism nor do they prove that a circulatory component is involved in the final damage incurred. However, since differences in flow occurred between the groups in spite of a similar derangement of cerebral energy state, and since postischemic perfusion rates may well be critical for recovery events, it seems justified that the problem is further explored.

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III

Indomethacin and Recirculation following Cerebral
Ischemia

by

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Abstract

In this study we examined the effect of pretreatment with the cyclo-oxygenase inhibitor indomethacin (10 mg.kg^{-1}) on local cerebral blood flow (CBF) in the recirculation period following complete and incomplete ischemia. Ischemia of 15 min duration was induced in lightly anaesthetized and artificially ventilated rats, and local CBF was measured with a ^{14}C -iodoantipyrine autoradiographic technique after recirculation periods of 5 min (complete and incomplete ischemia) and 60 min (incomplete ischemia in fed and fasted rats).

In the initial recovery period following complete ischemia indomethacin-treated animals showed a reduced incidence of perfusion defects of the "no-reflow" type. Perfused structures had somewhat higher flow rates than in untreated rats. A similar enhancement of immediate reflow was observed following incomplete ischemia provided that the structures in question had been severely ischemic. In structures that suffered only mild ischemia, the drug reduced postischemic CBF.

Measurement of l-CBF after 60 min of recirculation, following 15 min of incomplete ischemia, gave the following results. In fed animals, most of the structures that had suffered marked reduction in blood flow during ischemia showed increased CBF in indomethacin-treated animals, whereas the drug reduced CBF in structures that had suffered mild ischemia only. In fasted animals, though, indomethacin reduced CBF in both these types of structures, leaving CBF in structures with intermediate degrees of ischemia unchanged.

We conclude that the beneficial effect, if any, of indomethacin on postischemic circulatory disturbances manifests itself primarily by enhancing blood flow in the immediate recirculation period.

INTRODUCTION

It is now firmly established that cerebral ischemia of a certain minimal duration (5-10 min) leads to two types of postischemic circulatory disturbances (for documentation and references to the literature, see Kågström et al. 1982 a and b, submitted). First, at least following complete ischemia, failure of reperfusion may occur in centrally located brain structures ("no-reflow areas") even if a seemingly adequate cerebral perfusion pressure is restored at the termination of ischemia. With ischemia of 10-15 min duration, these perfusion defects subsequently disappear; however, following 30 min of ischemia they may persist, at least in part, for as long as 60-90 min. Second, whether the ischemia is complete or incomplete, recirculation is accompanied by a "delayed" hypoperfusion which may be regionally quite pronounced (flow rates down to 20-30% of control). Available evidence suggests that this secondary hypoperfusion may persist for 4-8 hours (Levy et al. 1979, Pulsinelli et al. 1982).

It cannot at present be excluded that failure of reperfusion and/or the appearance of a delayed hypoperfusion contribute to the final cell damage incurred after ischemia. It is natural, therefore, that attempts are being made to unravel the mechanisms responsible for these circulatory disturbances, and to seek measures that ameliorate them. In a recent study, Hallenbeck and Furlow (1979) reported that the cyclo-oxygenase inhibitor indomethacin significantly enhanced postischemic CBF in dogs subjected to 35 min of CSF compression ischemia. Control animals subjected to this insult showed low CBF values with areas of greatly diminished flow rates, as measured with a ^{14}C -iodoantipyrine autoradiographic technique 30 min following restoration of perfusion pressure. Pretreatment with indomethacin, or postischemic administration of indomethacin plus prostacyclin, enhanced reflow and completely abolished the zones of greatly impaired reperfusion. The authors concluded that an imbalance in the prostaglandin pathways is at least in part responsible for postischemic cerebral circulatory disturbances.

In monkeys (Pickard and MacKenzie 1973, Pickard et al. 1977), rats (Sakabe and Siesjö 1979, Dahlgren et al. 1981 a), and man (Wennmalm et al. 1981) indomethacin reduces "resting" CBF and dramatically curtails the CO_2 -responsiveness of the cerebral circulation.

However, neither the corresponding responsiveness to hypoxia (Sakabe and Siesjö 1979) nor the hyperemia accompanying severe hypoglycemia (Nilsson et al. 1981) and epileptic seizures (Ingvar et al. 1981) are affected by the drug. Recent results suggest that the effect of indomethacin on normocapnic and hypercapnic CBF is species dependent and not necessarily related to cyclo-oxygenase inhibition (Busija et al. 1982, Wennmalm et al. 1982, Siesjö and Wieloch 1982a). It cannot be excluded, though, that the beneficial effects of indomethacin in enhancing postischemic cerebral circulation is related to inhibition of prostaglandin (and/or thromboxane) formation; thus indomethacin shares with several other cyclo-oxygenase inhibitors the capacity to curtail formation of prostaglandins in the brain (Abdel-Halim et al. 1978, Gaudet et al. 1980).

Although the data reported by Hallenbeck and Furlow (1979) have received support by results on rabbits subjected to global ischemia of 10 min duration (Boulu et al. 1981) the effects of indomethacin on postischemic CBF have not been systematically explored. In the present experiments, we evaluated the effect of indomethacin on initial (5 min) postischemic flow rates following both complete and incomplete ischemia of 15 min duration, as well as on the delayed hypoperfusion as this occurs in fed and fasted animals after 60 min of recirculation, following 15 min of incomplete ("forebrain") ischemia.

METHODS

Operative, sampling, and analytical techniques

The experiments were performed on male Wistar rats (350-400 g). The animals had free access to tap water until operation. Fasted animals were withdrawn food (rat pellets) during the night before the experiment. Anaesthesia was induced with 3% halothane and, after tracheostomy, the animals were ventilated with a mixture of 0.7% halothane and 70% N₂O in oxygen during the subsequent operation. The femoral arteries, femoral veins, and one brachial artery were cannulated for continuous blood pressure recording, infusions, injections, and blood sampling. Goldplated copper screws were inserted into the skull bone for EEG recordings. The animals were given heparin (100 I.U. i.v.) and paralyzed with tubocurarine chloride (2 mg.kg⁻¹ i.v.). When the surgical procedure was completed the halothane supply was discontinued and ventilation (on 70% N₂O and 30% O₂) was adjusted to give arterial oxygen (PaO₂) and carbon dioxide (PaCO₂) tensions of about 100 and 33-40 mm Hg, respectively. Before induction of ischemia the animals were allowed a steady state period of 30 min.

Sampling of arterial blood was performed anaerobically in glass capillaries for pH and blood gas determinations. PaO₂, PaCO₂, and pH were measured with microelectrodes (Eschweiler and Co., Kiel, and Radiometer, Copenhagen), operated at 37°C, and corrections were made for the body temperature. Whole blood concentrations of glucose, lactate, and pyruvate were analysed after sampling of arterial blood directly into liquid nitrogen.

In experiments designed for studies of cerebral metabolites the brains were frozen in situ (Pontén et al. 1973) and then chiselled out and stored at -80°C until extraction. A fronto-parietal part was taken from each hemisphere, weighed and extracted at -22°C. The metabolites were analyzed with the specific, enzymatic, fluorometric techniques of Lowry and Passonneau (1972). Metabolites were expressed as $\mu\text{mol.g}^{-1}$ wet tissue.

Statistical comparison was performed with the use of Student's t-test; $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$.

Reversible complete compression ischemia

Complete ischemia was induced with the method described by Ljunggren et al. (1974) and Nordström et al. (1978 a). The intracranial pressure was rapidly increased to at least 40 mm Hg above the mean arterial blood pressure by infusion of an artificial CSF solution into the cisterna magna. The vasopressor response to increased intracranial pressure was minimized by i.v. infusion of Arfonad® (tri-metnaphan-camphor-sulphonate), sometimes supplemented by arterial bleeding. To restore the cerebral perfusion pressure we interrupted the cisternal infusion and normalized the blood pressure. A fall in the intracranial temperature during the ischemic period was avoided by placing a heating bulb at a predetermined distance from the head of the animal. Excessive systemic acidosis during the recirculation period was counteracted by repeated i.v. injections of small doses of 0.6 M sodium bicarbonate (total volume 1.0-1.5 ml per animal) and ventilation volume was increased to compensate for an increase in PaCO_2 .

Reversible incomplete forebrain ischemia

Induction of incomplete ischemia was performed by occlusion of both common carotid arteries with the use of rubber-coated clamps (Nordström et al. 1978 b). At the same time the mean arterial blood pressure was decreased to 50 mm Hg by arterial bleeding, and kept at that level during the ischemic period. We restored brain perfusion by removal of the clamps and normalized the blood pressure by reinfusion of blood. Aberrations in intracranial temperature and in plasma acid-base state was avoided as described under the previous heading

Measurement of local CBF (lCBF)

We measured local CBF with the technique described by Landau et al. (1955) and Sakurada et al. (1978). Modifications of procedures applied in this study have been described in previous publications (Abdul-Rahman et al. 1979, Ingvar et al. 1981). In brief, 30-50 μCi of ^{14}C -iodoantipyrine, dispensed in 0.5-1.0 ml of Krebs-Hensleit solution, was infused i.v. at a constant rate for 20, 30, or 45 sec. Blood samples were obtained from the brachial artery catheter before and at frequent intervals during the infusion (every second to fifth sec). At the end of the infusion period the animal was decapitated. If a reduced CBF was expected the infusion period was 45 sec and if a

high flow rate was expected the infusion period was decreased to 20 sec. Intervals between samplings were changed accordingly. Following decapitation the brain was removed from the skull (within 2 min) and immediately immersed in isopentane chilled to -55 to -65°C . The ^{14}C activity in brain tissue (per unit mass) was evaluated by autoradiography. Slices of brain ($20\text{ }\mu\text{m}$) were cut in a Leitz sledge microtome at -20°C and applied to an X-ray film which was then exposed for one week. A series of calibrated ^{14}C -standards was applied to each film together with the brain slices. The film was then evaluated manually with a densitometer (McBeth, 1 mm aperture).

Calculation of local CBF was performed as described by Sakurada et al. (1978). In spite of the adjustments of the rate of isotope infusion mentioned above, it was not possible to determine lCBF accurately at all flow rates. If ^{14}C activity in a structure became so high that one reached the steep part of the logarithmic curve which is obtained by plotting the ^{14}C activity in the brain tissue against the corresponding CBF values, we chose to consider values inaccurate when an estimated 2% error in the determination of ^{14}C activity corresponded to a 10% variation in lCBF, as evaluated in each individual animal. If activities were higher, lCBF was assigned a value determined at that "break point". This implies that lCBF values were somewhat underestimated in some structures with high flow rates.

Experimental groups

Local CBF was evaluated in four groups of rats, each comprising 6 animals, in which indomethacin (Confortid[®], Dumex, $10\text{ mg}\cdot\text{kg}^{-1}$) had been given i.v. after the termination of the operative procedure and at least 30 min before induction of ischemia. In one group, with fed rats, lCBF was measured after 5 min of recirculation following 15 min of complete compression ischemia. A second group with fed rats was exposed to incomplete ischemia for 15 min and lCBF was measured after 5 min of recovery. In two groups, one with fed and one with fasted rats, 15 min incomplete ischemia was induced and lCBF evaluated after 60 min of recirculation.

Two uninjected animals were exposed to 15 min of complete ischemia and lCBF was measured after 5 min of recirculation. They were added to a group of 6 animals with similar treatment, reported pre-

viously (Kågström et al. 1982 a, submitted).

The control values for lCBF were taken from a previous study (Kågström et al. 1982 a, submitted) as they were obtained by the same investigators, using the same techniques as in the present study. The control groups, including 7 fed and 5 fasted animals, consisted of animals kept artificially ventilated for periods corresponding to the duration of the experiments. Anaesthesia, as well as operative and sampling techniques, were the same as in the experimental groups.

Cerebral cortical metabolites were studied in 4 groups of fed rats, each comprising 4 animals. Two groups, one with uninjected and one with injected animals, were examined at the end of 15 min incomplete ischemia. Another two groups, one with uninjected and one with injected animals, were examined after 5 min of recirculation following 15 min of incomplete ischemia.

R E S U L T S

As described above, the present material consists of four groups of animals, given indomethacin (10 mg.kg^{-1}) about 30 min prior to induction of ischemia. In two groups of fed animals, complete or incomplete ischemia of 15 min duration was induced, and l-CBF measured after 5 min of recirculation. In the other two groups 15 min of incomplete ischemia was induced in fed or fasted rats, and l-CBF measured after 60 min of recirculation. The indomethacin-treated animals were studied by the same group of investigators, using techniques similar to those employed in non-treated animals (Kågström et al. 1982 a and b, submitted). The present results, obtained on indomethacin-treated animals, will therefore be compared to those described previously.

Physiological data

Table 1 gives body temperature and mean arterial blood pressure, as well as arterial blood gases and pH. A comparison with previous results shows that indomethacin did not affect the physiological response of the animals to complete and incomplete ischemia. It should be emphasized that this statement also applies to the postischemic arterial blood and cerebral perfusion pressures. Thus, also in indo

Table 1. Physiological parameters in control animals and in animals subjected to 15 min complete or incomplete ischemia after indomethacin injection.

	Temp. °C	MABP mm Hg	PCO ₂ mm Hg	PO ₂ mm Hg	pH	n=
Controls, fed animals	37.1±0.1	152±2	36.8±0.8	100±5	7.41±0.01	7
Controls, fasted animals	37.3±0.2	144±2	35.7±0.5	100±3	7.40±0.01	5
Complete isch. 15 ⁻ Recirc. 5 ⁻						
Preisch.	37.2±0.3	143±5	37.1±0.9	100±6	7.40±0.04	6
Recirc. 5 ⁻	37.0±0.3	153±6	39.7±2.9	115±14	7.41±0.08	6
Incompl.isch.15 ⁻ Recirc. 5 ⁻						
Preisch.	37.0±0.2	158±3	39.5±0.9	95±1	7.40±0.01	6
Recirc. 5 ⁻	36.9±0.1	145±2	44.9±1.7	109±6	7.20±0.01	6
Incompl.isch.15 ⁻ Recirc.60 ⁻ , fed animals						
Preisch.	37.2±0.2	153±4	38.8±1.2	101±4	7.40±0.02	6
Recirc. 15 ⁻	37.4±0.3	149±3	36.4±2.2	138±4	7.29±0.03	6
Recirc. 60 ⁻	37.2±0.2	133±2	37.1±1.2	113±6	7.42±0.02	6
Incompl.isch.15 ⁻ Recirc.60 ⁻ , fasted animals						
Preisch.	37.2±0.2	152±1	35.1±1.5	103±4	7.37±0.02	6
Recirc. 15 ⁻	37.4±0.2	143±3	29.9±1.6	144±12	7.38±0.03	6
Recirc. 60 ⁻	37.3±0.4	128±4	36.9±3.2	111±5	7.34±0.05	6

methacin-injected animals these pressures returned to normal 1-2 min following the termination of complete and incomplete ischemia.

Previous results on incomplete ischemia showed that "delayed" recovery CBF values were somewhat higher in fasted than in fed rats (Kågström et al. 1982 b, submitted). Since fed animals developed

Table 2. Blood glucose, lactate, and pyruvate concentrations before, during, and following 15 min incomplete ischemia.

Sampling time	n=	<u>Glucose</u>		<u>Lactate</u>		<u>Pyruvate</u>	
		Control	Indometh.	Control	Indometh.	Control	Indometh.
Preisch.	22	10.6 $\pm$ 0.9	10.5 $\pm$ 0.8	1.5 $\pm$ 0.1	1.5 $\pm$ 0.1	0.143 $\pm$ 0.007	0.143 $\pm$ 0.00
Ischemia 15'	16	19.0 $\pm$ 1.7	18.8 $\pm$ 1.2	9.6 $\pm$ 0.4	10.1 $\pm$ 0.5	0.260 $\pm$ 0.014	0.262 $\pm$ 0.011
Recovery 5'	4	13.9 $\pm$ 1.5	13.7 $\pm$ 1.0	10.3 $\pm$ 0.4	9.2 $\pm$ 1.4	0.455 $\pm$ 0.049	0.503 $\pm$ 0.076
Recovery 15'	4	11.2 $\pm$ 2.5	14.8 $\pm$ 0.9	6.8 $\pm$ 0.7	10.7 $\pm$ 0.6	0.461 $\pm$ 0.084	0.392 $\pm$ 0.042
Recovery 30'	4	14.0 $\pm$ 0.8	13.8 $\pm$ 1.6	4.4 $\pm$ 0.4	5.7 $\pm$ 0.4	0.321 $\pm$ 0.014	0.332 $\pm$ 0.027

The concentrations are expressed in $\mu\text{mol}\cdot\text{g}^{-1}$. Values are means $\pm$ SEM.

marked hyperglycemia during the ischemia, and accumulated more lactate in the fronto-parietal cortex than fasted animals, it became of interest to study whether indomethacin affected this response. As table 2 shows, pretreatment with indomethacin did not alter the response of fed animals to incomplete ischemia. Thus blood glucose, lactate, and pyruvate concentrations were similarly altered in control and indomethacin-injected animals. Tissue lactate concentrations were also similar. In uninjected animals, the concentrations were 21.3 ± 2.6 and $17.9 \pm 1.5 \mu\text{mol}\cdot\text{g}^{-1}$ at the end of the ischemia and after 5 min of recovery, respectively. In indomethacin-injected animals, the corresponding values were 19.3 ± 4.5 and $17.1 \pm 1.5 \mu\text{mol}\cdot\text{g}^{-1}$, respectively. We conclude that any effect of indomethacin on recovery CBF values was unrelated to changes in blood glucose concentrations and tissue lactate concentrations.

Local CBF

Table 3 gives means $\pm$ SEM for local CBF values in all groups for which flow rates were homogenous within the 24 structures analysed. The values have been compared to control data for fed and fasted animals, as reported previously. In the following, we will describe results on immediate and delayed flow rates under separate headings, and relate the data to those previously reported for un-

injected animals.

a. Local flow rates after 5 min recovery. The results obtained following complete and incomplete ischemia will be described separately.

The previous results on complete ischemia (Kågström et al. 1982 a, submitted) showed that flow rates after 5 min of recirculation were strikingly heterogenous, with CBF values varying not only between structures but also within structures. Furthermore, in 6 of 6 animals zones of no-reflow appeared in centrally located structures such as the caudoputamen, thalamus, and hippocampus. Since results varied considerably between animals, two additional animals were studied for comparison with the indomethacin-treated ones. In these, the appearance of no-reflow areas were somewhat less pronounced than in the

Table 3. Local CBF in control animals and in the recirculation period following 15 min complete or incomplete ischemia in indomethacin-injected animals

Structures	Controls	Controls	Compl.isch.	Incompl.isch.	Incompl.	Incompl.
	fed n=7	fasted n=5	Recirc.5 ⁻ n=6	Rec.5 ⁻ ,fed n=6	isch. Rec.60 ⁻ , fed,n=6	isch. Rec.60 ⁻ , fasted,n=6
Frontal cortex	2.03±0.08	2.05±0.15	4.20	4.01±0.76	0.36±0.04	0.42±0.03
Nucl. accumbens	1.50±0.10	1.60±0.04	3.44±0.65	4.34±0.62	0.55±0.07	0.54±0.04
Caudoputamen	1.41±0.09	1.56±0.07	2.90	4.19±0.61	0.51±0.18	0.56±0.04
Sensorimotor cortex	1.98±0.10	1.82±0.17	5.06	6.32±0.25	0.40±0.02	0.48±0.04
Septal nucleus	0.85±0.06	0.85±0.06	3.23±0.39	3.01±0.31	0.29±0.02	0.35±0.03
Globus pallidus	0.66±0.07	0.69±0.05	2.06	2.69±0.36	0.31±0.01	0.37±0.04
Thalamus	1.63±0.09	1.68±0.09	3.04	4.95±0.72	0.47±0.04	0.59±0.03
Hypothalamus	1.05±0.08	1.07±0.09	2.18±0.32	2.04±0.23	0.44±0.04	0.46±0.05
Habenula	1.64±0.12	1.67±0.07	4.53±0.57	3.11±0.80	0.71±0.04	0.92±0.12
Lateral thalamus	1.53±0.09	1.65±0.07	4.45	5.44±0.69	0.49±0.04	0.63±0.05
Parietal cortex	1.89±0.09	2.29±0.23	5.23	5.77±0.52	0.36±0.02	0.37±0.03
Piriform cortex	1.12±0.10	1.26±0.11	3.15±0.52	2.86±0.27	0.39±0.05	0.34±0.03
Cingulate cortex	1.33±0.12	1.27±0.10	4.93±0.55	4.48±0.65	0.37±0.03	0.36±0.03
Amygdala	1.06±0.09	1.20±0.07	3.92±0.64	3.71±0.27	0.35±0.03	0.40±0.03
Hippocampus	0.87±0.07	0.98±0.12	2.90	3.69±0.36	0.31±0.02	0.37±0.03
Lat. geniculate body	1.40±0.11	1.42±0.06	4.22±0.69	5.02±0.64	0.45±0.02	0.57±0.07
Red nucleus	1.45±0.10	1.49±0.06	3.74	1.85±0.39	0.64±0.04	0.78±0.10
Substantia nigra	1.01±0.07	1.03±0.07	4.30±0.57	2.84±0.32	0.62±0.10	0.58±0.08
Med. geniculate body	1.82±0.09	2.09±0.11	5.78±0.33	5.45±0.51	0.54±0.03	0.73±0.08
Superior colliculus	1.48±0.11	1.32±0.08	5.17±0.51	4.12±0.59	0.68±0.04	0.83±0.12
Auditory cortex	3.12±0.15	3.47±0.28	5.12±0.48	6.06±0.42	0.54±0.07	0.54±0.04
Inferior colliculus	1.99±0.09	1.79±0.17	4.38	5.59±0.69	1.17±0.10	1.40±0.29
Visual cortex	1.52±0.08	1.49±0.09	4.28±0.48	4.74±0.57	0.50±0.09	0.42±0.03
Cerebellum	0.82±0.06	0.81±0.07	3.26±0.82	1.20±0.37	0.47±0.03	0.55±0.00

Local CBF is expressed in ml.min⁻¹.g⁻¹. Values are means + SEM.
For structures in which perfusion defects occurred (following complete ischemia), mean values only are given.

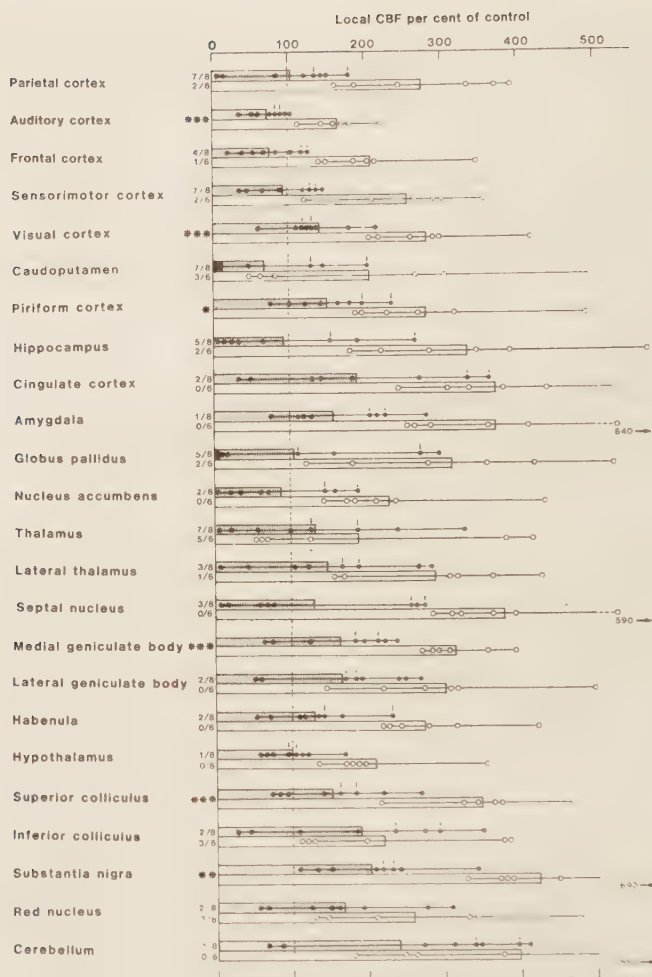


Fig. 1. Local CBF in per cent of control after 5 min of recirculation following 15 min of complete compression ischemia in uninjected (filled circles) and indomethacin-injected (open circles) rats. The notations to the left of the baseline indicate either the proportion of animals with perfusion defects of the no-reflow type in the structure in question or, for structures devoid of such defects, the degree of statistical significance of the difference between mean flow rates in uninjected and indomethacin-injected animals. Arrows indicate the values obtained in the two additional experiments on uninjected animals (see results).

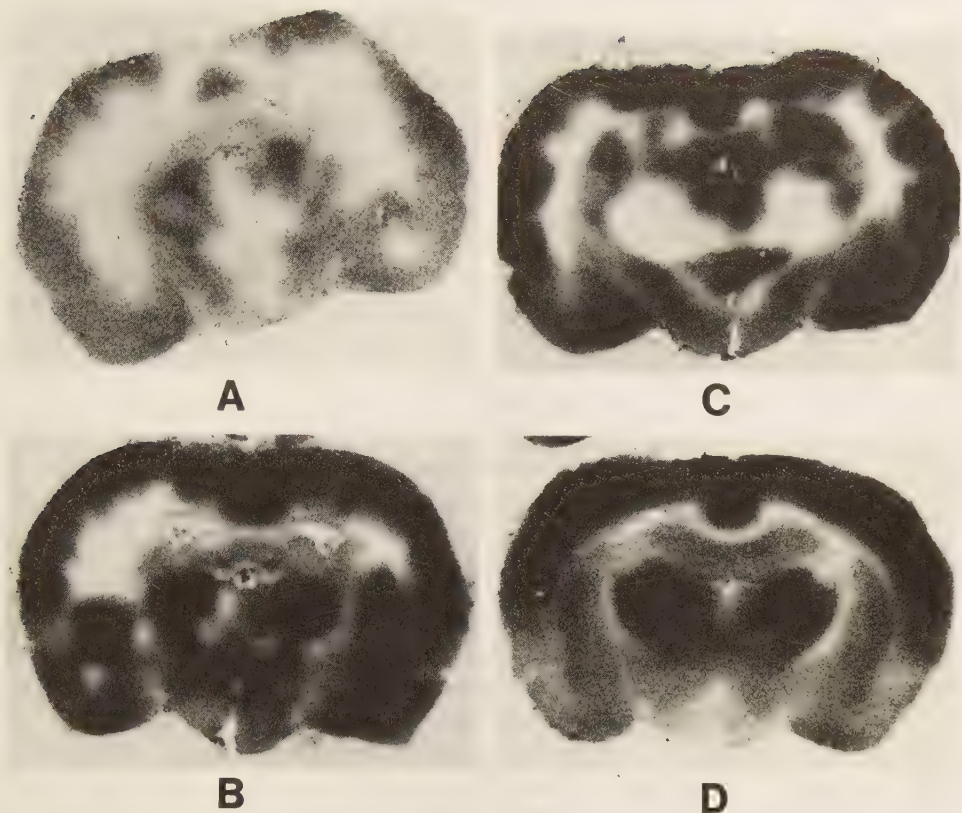


Fig. 2. Autoradiographic pictures of coronal brain sections from the thalamus/parietal cortex region after 5 min of recirculation following 15 min of complete compression ischemia. Pictures from uninjected (left) and indomethacin-injected (right) animals were chosen from the experiments with the most (top) and least (bottom) pronounced extensions of perfusion defects in the respective groups.

majority of the other six; nonetheless, the CBF values added were within the range of previously published ones (see arrows in fig. 1 below).

In fig.1, 1-CBF values have been compared for non-injected and indomethacin-injected animals. The results depicted in this figure, and those illustrated in fig. 2, demonstrate that pretreatment of the animals with indomethacin improved reflow. Thus, in the indomethacin-injected group, one animal had no perfusion defects at all (fig. 2, D), four had only small no reflow zones, mainly localized to the thalamus and the caudoputamen, while only one had quite large perfusion defects (fig 2, C). Furthermore, indomethacin-treated

animals had higher flow rates in perfused parts of the structures with no-reflow zones (see mean values). In six areas devoid of no-reflow zones in treated as well as untreated groups, it was possible to substantiate this effect on reperfusion. Thus, in all these structures the mean flow rates were significantly higher in indomethacin-injected than in uninjected animals ($p < 0.05$, fig. 1).

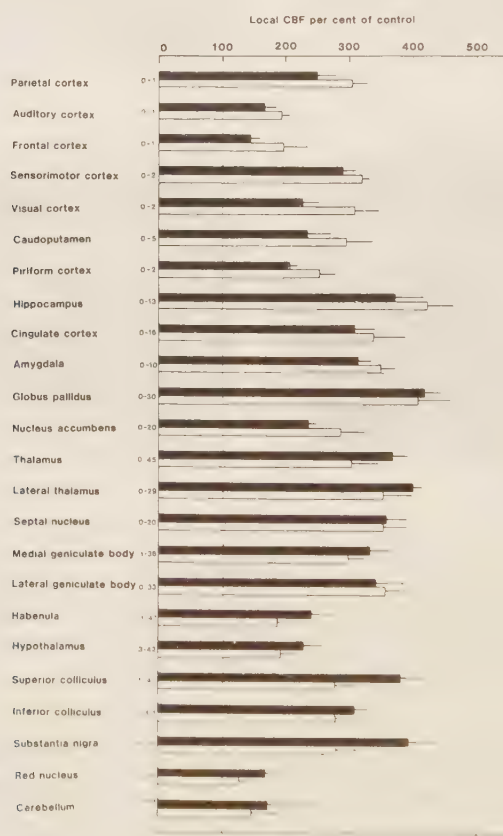


Fig. 3. Local CBF in per cent of control after 5 min of recirculation following 15 min of incomplete ischemia in uninjected (shaded columns) and indomethacin-injected (unshaded columns) animals. Values are means $\pm$ SEM. The figures to the left of the baseline indicate the range of flow rates in per cent of control in the structure during ischemia with the present model (cf Kågström et al. 1982 b, submitted). The differences between mean flow rates in uninjected and indomethacin-injected animals were not statistically significant in any of the structures examined.

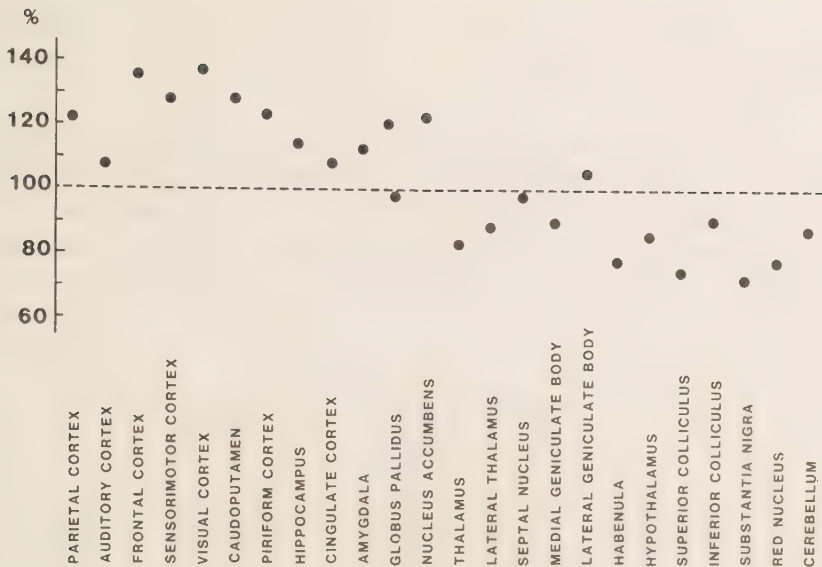


Fig. 4. Mean local CBF rates in indomethacin-injected animals after 5 min of recirculation following 15 min of incomplete ischemia, expressed in per cent of the corresponding mean flow rates in uninjected animals, treated similarly.

Following incomplete ischemia, zones of no-reflow do not appear (Kågström et al. 1982 b, submitted). It was possible, therefore, to calculate means $\pm$ SEM and to compare statistically uninjected and indomethacin-injected animals. In fig. 3, the structures have been listed in the order of decreasing severity of ischemia. Although the values were not statistically different the results suggest that indomethacin somewhat improved reflow in severely ischemic structures and lowered CBF in those with less severe or only moderate ischemia. This result seems even more probable when one inspects the data of fig. 4 which gives CBF values in indomethacin-treated animals in per cent of those measured in uninjected ones.

From the results presented, we conclude that indomethacin does not curtail the reactive hyperemia that ensues upon a 15 min period of complete, or severe incomplete ischemia. Rather, the drug seems to diminish the appearance of no-reflow zones, and to enhance

postischemic perfusion rates. However, in structures which suffer only moderate flow reductions during ischemia CBF values are reduced during reperfusion.

b. Local flow rates after 60 min of reperfusion. When the present results are considered, it should be recalled that, following incomplete ischemia, a rough correlation exists, at the local level, between the magnitude of the delayed hypoperfusion and the severity of the preceeding ischemia; furthermore, fed animals show a more pro-

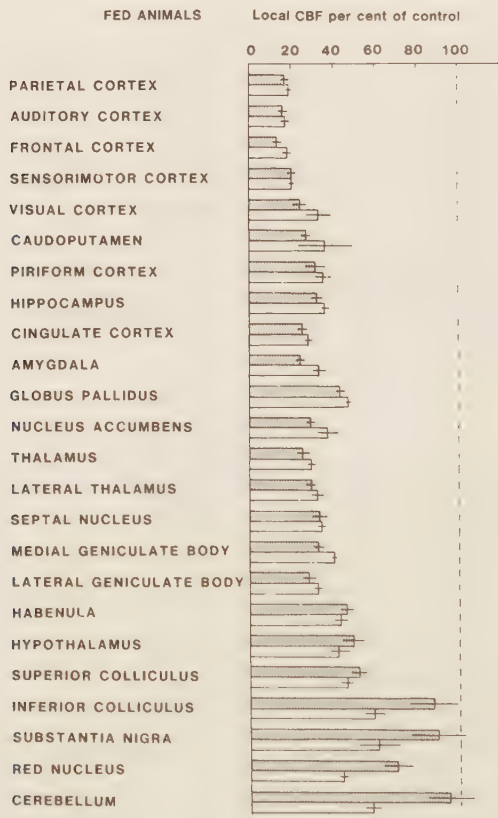


Fig. 5. Local CBF in per cent of control in fed rats after 60 min of recirculation following 15 min of incomplete ischemia, in uninjected (shaded columns) and indomethacin-injected (unshaded columns) animals. Values are means + SEM. The differences between mean flow rates in uninjected and indomethacin-injected groups were statistically significant in the amygdala, inferior colliculus and cerebellum ($p<0.05$), as well as in the red nucleus ($p<0.01$).

nounced reduction in CBF than fasted ones (Kågström et al. 1982 b, submitted).

Fig. 5 shows that, in fed animals, indomethacin seemed to somewhat enhance recirculation in structures whose flow rates were markedly reduced during the ischemia. Thus, in all such structures except one (sensorimotor cortex) mean flow rates were higher in indomethacin-treated animals than in uninjected ones. However, this

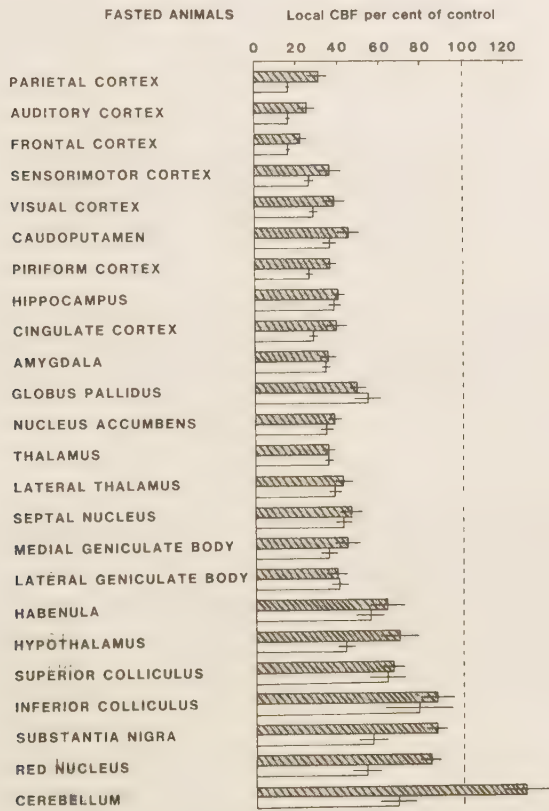


Fig. 6. Local CBF in per cent of control in fasted rats after 60 min of recirculation following 15 min of incomplete ischemia, in uninjected (shaded columns) and indomethacin-injected (unshaded columns) animals. Values are means + SEM. The differences between mean flow rates in uninjected and indomethacin-injected animals were statistically significant in the cingulate cortex, hypothalamus, and substantia nigra ($p < 0.05$), as well as in the red nucleus and cerebellum ($p < 0.01$).

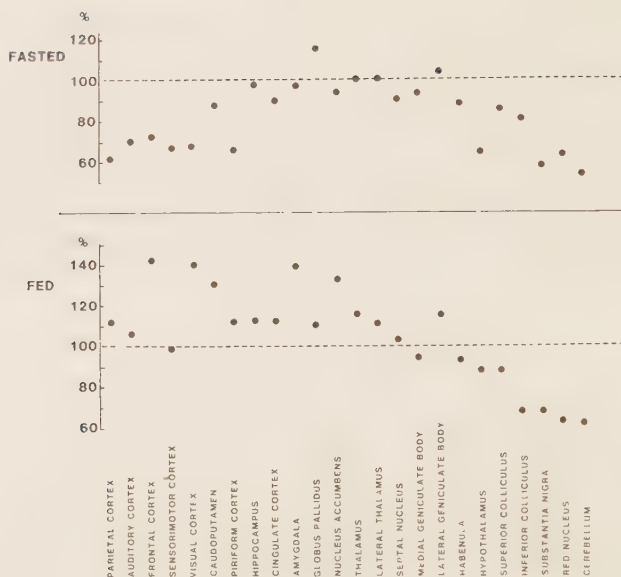


Fig. 7. Mean local CBF rates in indomethacin-injected animals, after 60 min of recirculation following 15 min of incomplete ischemia, in per cent of mean flow rates in uninjected animals, treated similarly. Fasted (top) and fed (bottom) animals.

suggested relationship was lost in fasted animals (fig. 6). In fact, in these indomethacin seemed to reduce CBF in a relatively uniform way. The relationships obtained are more apparent when flow rates in indomethacin-injected animals are plotted against those recorded in uninjected animals (fig. 7).

DISCUSSION

We will base our discussion of the results presented on three previous findings. These relate to (1) the arachidonic acid cascade elicited by an ischemic event, (2) the documented circulatory effects of indomethacin, and (3) the effect of cyclo-oxygenase inhibitors in modulating the effects of ischemia on the brain.

It is now well documented that ischemia of a severity that disrupts energy balance at the cellular level leads to accumulation of

free fatty acids (FFA) in the brain, the largest relative increase occurring in the concentration of arachidonic acid (Bazan 1970, 1976, Marion and Wolfe 1979, see also Gardiner et al. 1981), and that FFA concentrations only slowly decrease to control values during recirculation (Rehncrona et al. 1980, 1982, Yoshida et al. 1980). In the postischemic period, therefore, conditions are at hand for a cascade of oxidative reactions that are triggered by an increased arachidonic acid content. It was shown by Gaudet and Levine (1979) that massive accumulation of prostaglandins occur in this period, an accumulation that could be aborted by pretreatment with several cyclo-oxygenase inhibitors, including indomethacin (Gaudet et al. 1980). It seems clear that the products formed secondary to accumulation of arachidonic acid include all the major prostaglandins as well as thromboxane A_2 (Gaudet et al. 1980) and the lipoxygenase product 12 HETE (Sautebin et al. 1978, Spagnuolo et al. 1979), but it has never been documented that leucotrienes are formed as well (see Wolfe 1981, Siesjö and Wieloch 1982b). Nevertheless, there are reasons to believe that potentially toxic substances other than prostaglandins are formed, some of which have free radical character. Thus, free radicals are produced at the endoperoxide stage of the cyclo-oxygenase sequence, and evidence exists that such radicals also are formed nonenzymatically from arachidonic acid. One possible consequence of such production is "suicidal" inactivation of key enzymes, e.g. of prostacyclin synthetase (see Egan et al. 1976, Lands 1979). It is clear, therefore, that conditions are at hand for a potentially injurious sequence of postischemic reactions that may involve an imbalance between the vasoconstrictory and proaggregatory thromboxane A_2 and the vasodilatory and antiaggregatory prostacyclin, as well as free radical damage to vascular and parenchymal cells.

As stated in the introduction, indomethacin reduces CBF and curtails the CO_2 responsiveness in at least three species without interfering with the the dilatation encountered in hypoxia, hypoglycemia, and epileptic seizures (for review see Pickard 1981). However, the lack of circulatory effects of indomethacin in e.g. cats (Busija et al. 1982), and the fact that other cyclo-oxygenase blockers do not reduce normo- or hypercapnic CBF in man (Wenmalm et al. 1982) or rats (Wieloch et al., in preparation), suggest that the circulatory effects of indomethacin mentioned above are unrelated to cyclo-oxygenase

inhibition. Nonetheless, indomethacin is an efficient blocker of brain cyclo-oxygenase (Abdel-Halim et al. 1978), Gaudet et al. 1980), and it is tempting to conclude, therefore, that the effect of the drug in enhancing postischemic reperfusion (Hallenbeck and Furlow 1979) is due to cyclo-oxygenase inhibition (however, see below). Some evidence also exists that indomethacin can improve neurological recovery following ischemia (Gaudet and Levine 1979). It should be noted, though, that the effect of indomethacin is not necessarily beneficial in all ischemic situations, and that the adverse effects of an arachidonic acid cascade may be due to other compounds than cyclo-oxygenase products. For example, Branston et al. (1981) have reported that indomethacin can exaggerate edema formation following middle cerebral artery occlusion, and the induction of edema in brain tissue by arachidonic acid was not alleviated by indomethacin (Chan and Fishman 1978, 1980). These and other results (Kontos et al. 1980) indicate that adverse circulatory effects of an arachidonic acid cascade may entail production of toxic free radicals (see also Sano et al. 1980).

In this study pretreatment of animals with indomethacin seemed to enhance blood flow in the immediate recirculation period, and to diminish the appearance of no-reflow zones. Due to the pronounced interanimal variability in the 5 min recirculation $1\text{-CBF}'$ values, this conclusion rests on results that are difficult to prove statistically. However, apart from the fact that indomethacin-treated animals showed less extensive no-reflow zones, perfused areas had higher flow rates. Furthermore, higher flow rates in indomethacin-injected animals were also noted following incomplete ischemia. We tentatively conclude, therefore, that indomethacin promotes reflow following both complete and incomplete ischemia. It should be noted that the results unequivocally demonstrate that the drug does not curtail reactive hyperemia following an ischemic episode (cf results in hypoxia, hypoglycemia, and epileptic seizures).

The present results, notably those demonstrating that indomethacin reduced the incidence of no-reflow areas, fail to support the notion that the effect of the drug was to curtail formation of prostaglandins or thromboxanes in the immediate recirculation/reoxygenation period. Thus, since flow was not resumed in these areas it is difficult to argue that resupply of oxygen gave rise to rapid oxidation, via the cyclo-oxygenase step, of the arachidonic acid accumulated.

Possibly, the beneficial effect of indomethacin was due to its pre-ischemic actions, e.g. in reducing aggregation of thrombocytes and their adhesion to vascular endothelium. If so, one may envisage that drugs with such actions enhance reflow by reducing the hindrance to recirculation at the capillary level.

In the previous report (Kågström et al. 1982 b, submitted) we noted that, following incomplete ischemia, hyperemia seemed to be more pronounced in the structures that had been only moderately ischemic than in those exposed to severe ischemia (cf untreated animals in fig. 3). Since the potential for lactate accumulation in the brain tissue is greater in the former, we speculated that a correlation may exist between the degree of tissue acidosis incurred by ischemia and the amplitude of ensuing postischemic hyperemia. However, in indomethacin-treated animals the difference in postischemic flow rates between severely ischemic and less severely ischemic animals is less evident (fig. 3) and if the effect of the anesthetic procedure (Kågström et al. 1982 a, submitted, Dahlgren et al. 1981 b) is taken into account and comparison is made with unanesthetized animals, the difference is abolished (fig. 8). Furthermore, following complete ischemia in indomethacin-injected animals, with presumably even less accumulation of tissue lactate, postischemic hyperemia reaches approximately the same levels as following incomplete ischemia. (fig. 1 and 3). Since we have shown that treatment with indomethacin has no effect on cortical tissue lactate levels during and after ischemia, our results lend no support to the assumption that the amplitude of hyperemia following ischemia is related to the degree of brain tissue lactate concentration.

Although the drug also somewhat ameliorated the delayed hypoperfusion in fed animals, the results obtained on fasted animals showed that indomethacin did not generally improve postischemic perfusion. At first sight, these results seem at variance with those reported by Hallenbeck and Furlow (1979). However, the difference in results may be more apparent than real. Thus, these authors induced complete ischemia of 35 min duration, and measured CBF at a single postischemic period (30 min). It seems that, with such extended ischemic period, the appearance of zones of greatly impaired tissue perfusion represents a lingering no-reflow phenomenon rather than a secondary deterioration of CBF (Kågström et al. 1982 a, submitted).

Thus if we assume that pretreatment with indomethacin had the effect of improving immediate reflow, our own results are in line with those of Hallenbeck and Furlow (1979).

The present results demonstrate that indomethacin had different regional effects depending on whether structures were severely or moderately ischemic. This is particularly evident if one considers the results obtained in fed animals after 5 min of recirculation following

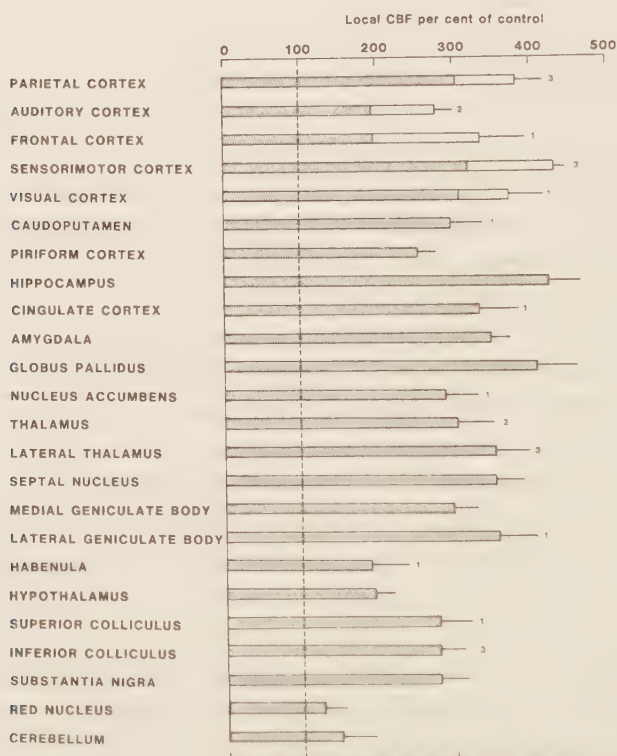


Fig. 8. Local CBF in per cent of control after 5 min of recirculation following 15 min of incomplete ischemia in indomethacin-injected rats. Values are means $\pm$ SEM. Shaded columns represent mean values obtained on comparison with N₂O-anesthetized control animals, and the unshaded part of a column corresponds to the increase in relative flow rate obtained when comparison is made with unanesthetized animals (see text). The figure to the right of a column indicates the number of experiments in the group in which the flow rate is somewhat underestimated in the structure in question (cf Methods).

incomplete ischemia (fig. 3) but, with the exception of parietal, sensorimotor, and auditory cortex, the same relationship was observed after 60 min of recirculation (fig. 5). The results depicted in these graphs make it tempting to conclude that indomethacin enhances reflow in proportion to the severity to the preceeding ischemia. However, the relationship did not apply to the fasted animals. In these, indomethacin reduced CBF in severely ischemic and in virtually non-ischemic structures but had no effect on structures with intermediate degrees of ischemia. Although no explanation can be afforded for this finding, the results render untenable the general conclusion that indomethacin ameliorates the delayed hypoperfusion that develops after ischemia. Obviously, this type of postischemic circulatory disturbance must be due to other mechanisms than those related to enhanced cyclooxygenase activity. It remains to be shown whether other oxidative products of arachidonic acid are responsible, or if the mechanisms responsible are unrelated to phospholipid degradation during ischemia.

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IV

Cerebral Circulatory Responses to Hypercapnia and
Hypoxia in the Recovery Period following Complete and
Incomplete Cerebral Ischemia in the Rat

by

Erik Kågström, Maj-Lis Smith, and Bo K. Siesjö

Abstract

In this study we wanted to examine the reactions of cerebral vessels to hypercapnia and hypoxia during the recovery period following cerebral ischemia. We used ventilated, lightly anaesthetized rats and induced complete ischemia by CSF compression, incomplete ischemia by bilateral carotid occlusion combined with hypotension. After 15 min of ischemia and 60 min of recirculation the animals were rendered hypercapnic or hypoxic for 2-3 min and local CBF was then measured autoradiographically with ^{14}C -iodoantipyrine.

Following complete ischemia vascular CO_2 responsiveness was abolished or attenuated in most structures analysed. However, there was a considerable interstructural heterogeneity. For example, in the cerebellum and the red nucleus flow rates were observed which approached values obtained in hypercapnic control animals, whereas CO_2 responsiveness was abolished in several cortical areas and hippocampus.

The response to CO_2 following incomplete ischemia varied considerably. In the cerebral cortices areas with low flow rates were often mixed with hyperemic zones, and in most structures that had very low flow rates during ischemia, CO_2 responsiveness was lost or grossly attenuated. Structures that had suffered moderate or only mild ischemia had better retained or completely preserved CO_2 response.

The cerebrovascular reaction to hypoxia was found to be attenuated in most, but not abolished in any of the structures examined. In general, the vascular response to hypoxia was better preserved than that to hypercapnia. Reactivity was similar following complete and incomplete ischemia. As observed during hypercapnia, there were pronounced interstructural variations with considerable increases in flow rates e.g. in the substantia nigra and the cerebellum.

INTRODUCTION

It is currently debated whether postischemic circulatory disturbances contribute to the final neurological deficit and/or neuronal cell damage that result from transient brain ischemia (for discussion and further literature, see Miller et al. 1980a, Siesjö 1981, Pulsinelli et al. 1982 a and b). Two such disturbances have been described. Some years ago, much interest was focussed on the possibility that zones of impaired perfusion persist for some time in the recirculation period, provided that the preceeding ischemia exceeds 5-7 min ("no-reflow phenomenon"), and it was speculated that the local perfusion defects were due to increased viscosity of stagnating blood and some narrowing of capillary lumina (see Fischer et al. 1977, 1979). However, many subsequent studies, in which an adequate post-ischemic perfusion pressure was secured, revealed that recirculation led to a transient increase in CBF ("reactive hyperemia") but that a secondary reduction in tissue perfusion ("delayed hypoperfusion") subsequently developed (Hossmann et al. 1973, Nemoto et al. 1975, Levy et al. 1979, Jackson et al. 1980, Miller et al. 1980 a).

The cause of the delayed hypoperfusion is not known. However, since the possibility remains that it contributes to "maturation" of the cell damage (see Ito et al. 1975, Pulsinelli et al. 1982 b) efforts have been made to unravel the underlying mechanisms, and to seek measures that ameliorate it. It has been speculated by some that the hypoperfusion reflects vasoparalysis, e.g. due to tissue acidosis (Fazekas and Alman 1964, Nemoto et al. 1975). However, most workers now favour the view that the postischemic reduction i CBF represents vasoconstriction of unknown cause (e.g. Hossmann et al. 1977, Miller et al. 1980 a). Two previous studies showed that vascular autoregulation was preserved in the postischemic period but that the CO₂ responsiveness was abolished (Hossmann et al. 1973, Nemoto et al. 1975), and the vascular unreactivity was also demonstrated by the fact that a number of vasoactive drugs failed to enhance CBF (Hossmann et al. 1973, Takagi et al. 1977). Hossmann et al. (1973) observed that the CO₂ reactivity was attenuated after ischemia of 3.5 min duration and abolished when the ischemic period was prolonged to 10 min. In a recent study (Miller et al. 1980 b) the authors induced ischemia of

only 5 min duration and measured postischemic cortical CBF at multiple sites, using hydrogen electrodes. With this protocol, hypercapnia was found to moderately increase CBF (*i.e.* to alleviate the delayed hypoperfusion) at most of the electrode sites. All these observations concerning CO₂ reactivity are based on models of complete ischemia and, except those of Miller et al. (1980b), with measurement of global CBF only.

In two previous articles we described circulatory events during recovery from 15 min of complete and incomplete ischemia in lightly anesthetized, artificially ventilated rats, using autoradiographic techniques for measuring local CBF (Kågström et al. 1982 a and b, submitted). The results of these studies confirmed previous data in showing that transient ischemia of that duration usually is followed by hyperemia, in turn succeeded by a reduction in CBF. However, in the initial recovery phase of complete ischemia, whether induced by CSF compression or by four-vessel-occlusion combined with hypotension, a no-reflow phenomenon occurred in centrally located brain structures. With ischemia of 15 min duration, though, the perfusion defects disappeared when the recirculation period was extended to 60 min. At that time, the autoradiographic technique used revealed pronounced interstructural heterogeneities in delayed hypoperfusion. For example, following complete ischemia, many structures including the cerebral cortices had flow rates below 1/3 of control, but in some regions, particularly the cerebellum, CBF values were close to normal.

The objective of the present study, which is solely concerned with the delayed hypoperfusion, was to explore if local differences exist in vascular reactivity and if loss of reactivity bears any relationship to the severity of the preceding ischemia.

To meet these objectives we studied flow patterns after 60 min of recirculation, following 15 min of both complete and incomplete ischemia, and measured local CBF autoradiographically after a 2-3 min period of hypercapnia or hypoxia.

METHODS

Operative, sampling, and analytical techniques

The experiments were performed on male Wistar rats (350-400 g) of a S.P.F. strain (Møllegaard Avlslaboratorium, Copenhagen). The animals had free access to water and rat pellets until operation. Anaesthesia was induced with 3% halothane. Following tracheostomy the rats were paralyzed with tubocurarine chloride ($2 \text{ mg} \cdot \text{kg}^{-1}$ i.v.) and artificially ventilated with a mixture of 0.7% halothane in 30% O_2 and 70% N_2O during the surgical procedure. The femoral arteries, femoral veins, and one brachial artery were cannulated for continuous blood pressure recording, infusions, injections and blood sampling. Two copper screws were inserted in the skull bone for EEG recordings. When the operation was finished the animals were given heparin (100 I.U. i.v.) and ventilation was adjusted to give arterial oxygen and carbon dioxide tensions of about 100 and 34-40 mm Hg, respectively. Before induction of ischemia the animals were allowed a steady state period of at least 30 min.

Blood gases were determined after anaerobical sampling of arterial blood in glass capillaries. Arterial oxygen (PaO_2) and carbon dioxide (PaCO_2) tensions and pH were measured with microelectrodes (Eschweiler and Co., Kiel, and Radiometer, Copenhagen), operated at 37°C , with corrections for body temperature.

Statistical evaluation was performed using Student's t-test, $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$.

Reversible complete cerebral ischemia

Complete ischemia was accomplished with the CSF compression method described by Ljunggren et al. (1973). The intracranial pressure was raised to a level at least 30 mm Hg above the mean arterial blood pressure by infusion of a mock CSF solution into the cisterna magna through a double-barrelled needle which allowed continuous recording of the pressure in the cisterna magna. A pressor response was counteracted by previous i.v. infusion of Arfonad® (trimethaphan-camphor-sulphonate). Circulation to the brain was restored by discontinuing the CSF-infusion and blood was infused i.v. to normalize the blood pressure.

Reversible incomplete forebrain ischemia

Incomplete ischemia was induced with the method described by Nordström et al. (1978). The common carotid arteries were occluded bilaterally with rubber-coated clamps and at the same time the mean arterial blood pressure was decreased to 50 mm Hg and kept at that level during the ischemia with a servosystem which allowed automatic extraction and reinfusion of blood. A heating bulb was placed at a predetermined distance from the head of the animal to avoid a fall in the intracranial temperature during the ischemic period. At the end of ischemia the clamps on the carotid arteries were removed and the blood pressure was normalized by reinfusion of blood. During the recirculation period repeated i.v. injections of 0.6 M sodium bicarbonate were made to counteract systemic acidosis and the ventilation volume was increased to compensate for an increase in PaCO_2 .

Measurement of local CBF (lCBF)

Measurement of local CBF was performed with the technique described by Landau et al. (1955) with modifications of Sakurada et al. (1978). Applications of the procedure used in this study have been reported in previous publications from the laboratory (Abdul-Rahman et al. 1979, Ingvar et al. 1981). In summary, 20–30 μCi of ^{14}C -iodoantipyrine in 0.5–1.0 ml of Krebs-Hensleit solution was infused i.v. with a constant rate for 30 or 45 sec. Blood samples were taken from the brachial artery before and every 3rd or 4th sec during the infusion. At 30 (or 45) sec the animal was decapitated. The brain was then removed from the skull and immersed in isopentane chilled to -55 to -65°C . The ^{14}C activity in brain tissue was evaluated by autoradiography. Twenty μm thick slices of brain were cut in a Leitz sledge microtome at -20°C and applied to an X ray film which was then exposed for one week. A series of calibrated ^{14}C -standards was applied to each film together with the cut brain slices. The film was evaluated manually with a densitometer (McBeth, 1 mm aperture). At least 6 readings were made in each structure, in 3 adjacent slices. The means of the values thus obtained were used for the calculation of CBF which was performed as described by Sakurada et al. (1978).

Induction of hypercapnia and hypoxia

When this study was started we considered that measurement of lCBF after a 5-6 min exposure to hypercapnia and hypoxia would provide reliable information of the reactivity of cerebral vessels during the period of delayed hypoperfusion following ischemia. However, it soon became clear that following complete ischemia, the normal vasopressor response to hypercapnia and hypoxia was often inversed. This was true particularly when hypoxia was induced. To be able to carry the experiments through in steady states at acceptable blood pressure levels we had to shorten the period of exposure to 2-3 min. However, in preliminary experiments with the use of the venous outflow technique described by Nilsson and Siesjö (1982), we had found that maximal effects on flow rates were obtained within 30-60 sec after the commencement of hypercapnia/hypoxia (data not shown). This observation is substantiated by the results of previous studies on hypoxia (Borgström et al. 1975) and hypercapnia (Nilsson et al. 1981). Consequently we induced hypercapnia by adding 10% carbon dioxide to the inhalation mixture and measured lCBF after 2-3 min. Hypoxia was induced by reducing the oxygen concentration in the inhalation mixture from 30% to 6% and adding 24% nitrogen. The hypercapnic control animals were exposed to 5-6 min of hypercapnia before lCBF measurement. This experimental period was chosen since the values for hypoxic control animals were taken from a concurrent study (Smith et al. 1982, in prep.) in which the animals had been exposed to moderate hypoxia (about 25 mm Hg) for 5-6 min.

Experimental groups

There are 5 experimental groups in this study, each comprising 6 animals. Two groups were exposed to 15 min complete ischemia and after 60 min of recirculation lCBF was measured after a 2-3 min exposure to hypercapnia in one group and hypoxia in the other. In two groups incomplete ischemia was induced and the animals were otherwise treated similarly.

Hypercapnic control values for lCBF were obtained from a group of 6 animals which had been kept artificially ventilated for periods corresponding to the experiments and exposed to 5-6 min of hypercapnia followed by measurement of lCBF.

R E S U L T S

As stated, the present material consists of 5 groups, four with a preceding period of ischemia, and one hypercapnic control group. A concurrent study from the laboratory gives data on ICBF changes in hypoxia of comparable severity (Smith et al. 1982, in

Table 1. Physiological parameters in normocapnic and hypercapnic control animals and in animals exposed to hypercapnia or hypoxia after 60 min of recirculation following 15 min of complete or incomplete ischemia.

	Temp. °C	MABP mm Hg	PCO ₂ mm Hg	PO ₂ mm Hg	pH	n=
Normocapnic controls	37.1±0.1	152±2	100±5	36.8±0.8	7.41±0.01	7
Hypercapnic controls						
Before hypercapnia	37.1±0.1	151±5	107±4	38.6±0.8	7.48±0.04	6
During hypercapnia	36.9±0.1	163±4	111±4	73.0±4.5	7.24±0.04	6
Complete isch.						
Before ischemia	37.4±0.1	155±4	107±4	35.2±0.7	7.44±0.01	6
Recirc. 15"	37.0±0.1	141±4	124±3	32.9±0.5	7.45±0.02	6
Recirc. 60"	37.3±0.2	148±4	118±5	35.4±1.5	7.39±0.03	6
During hypercapnia	37.3±0.2	144±6	115±5	72.4±3.9	7.15±0.03	6
Incomplete ischemia						
Before ischemia	37.4±0.1	150±3	104±1	39.4±0.5	7.40±0.01	6
Recirc. 15"	37.8±0.2	148±4	124±6	38.9±1.2	7.30±0.02	6
Recirc. 60"	37.2±0.2	144±5	127±4	34.7±1.7	7.41±0.05	6
During hypercapnia	37.2±0.2	155±6	129±3	71.5±0.4	7.19±0.05	6
Complete ischemia						
Before ischemia"	37.2±0.2	154±7	100±3	36.7±1.2	7.43±0.01	6
Recirc. 15"	37.2±0.3	143±4	122±4	31.1±0.9	7.46±0.01	6
Recirc. 60"	37.2±0.2	149±7	117±5	35.1±1.2	7.41±0.02	6
During hypoxia	37.2±0.2	108±10	23.8±1.2	31.4±1.0	7.42±0.04	6
Incomplete ischemia						
Before ischemia	37.5±0.2	149±4	99±5	36.6±1.6	7.44±0.01	6
Recirc. 15"	37.5±0.1	146±4	119±7	36.4±1.6	7.31±0.04	6
Recirc. 60"	37.5±0.1	132±5	116±2	35.2±1.1	7.39±0.03	6
During hypoxia	37.5±0.1	133±9	27.2±3.5	33.5±1.2	7.38±0.02	6

Values are means ± SEM

Table 2. Local CBF in normocapnic and hypercapnic control animals and after 60 min of recirculation following 15 min of complete or incomplete ischemia and exposure to hypercapnia or hypoxia.

Structures	Controls	Controls	Compl.isch.	Compl.isch.	Incompl.	Incompl.
	normocapn. n=7	hypercapn. n=6	hypercapn. n=6	hypoxia n=6	isch. hypercapn. n=6	isch. hypoxia n=6
Frontal cortex	2.03±0.08	4.67±0.29	0.97±0.13	1.48±0.16	1.55±0.68	1.50±0.29
Nucl. accumbens	1.50±0.10	4.18±0.37	0.94±0.08	1.47±0.18	0.92±0.15	1.49±0.23
Caudoputamen	1.41±0.09	3.77±0.24	1.11±0.30	1.43±0.19	0.79±0.10	1.25±0.18
Sensorimotor cortex	1.98±0.10	5.75±0.50	1.36±0.20	1.95±0.15	1.72±0.48	2.18±0.44
Septal nucleus	0.85±0.06	3.21±0.26	0.82±0.09	1.60±0.14	0.89±0.14	1.50±0.28
Globus pallidus	0.66±0.07	2.61±0.20	0.86±0.21	1.45±0.13	0.92±0.13	1.40±0.21
Thalamus	1.63±0.09	4.77±0.30	2.29±0.38	2.24±0.25	2.45±0.57	2.20±0.39
Hypothalamus	1.05±0.08	3.14±0.21	2.02±0.53	2.24±0.19	2.34±0.46	2.25±0.39
Habenula	1.64±0.12	5.23±0.44	1.81±0.33	3.23±0.40	2.92±0.40	3.19±0.63
Lateral thalamus	1.53±0.09	4.06±0.33	1.73±0.20	2.53±0.26	2.81±0.45	2.58±0.40
Parietal cortex	1.89±0.09	5.50±0.36	1.39±0.20	2.14±0.23	2.05±0.62	2.20±0.48
Piriform cortex	1.12±0.10	3.11±0.12	0.70±0.12	1.05±0.12	0.63±0.13	1.09±0.17
Cingulate cortex	1.33±0.17	3.90±0.36	0.96±0.10	1.87±0.22	1.62±0.43	1.95±0.39
Amygdala	1.06±0.09	3.09±0.10	0.96±0.10	1.35±0.11	1.26±0.21	1.32±0.19
Hippocampus	0.87±0.07	2.84±0.27	0.51±0.05	1.37±0.14	0.82±0.19	1.09±0.15
Lat. geniculate body	1.40±0.11	3.97±0.27	1.21±0.20	1.98±0.14	2.18±0.40	2.31±0.49
Red nucleus	1.45±0.10	3.49±0.22	3.19±0.59	2.83±0.36	3.28±0.38	3.44±0.28
Substantia nigra	1.01±0.07	3.21±0.23	2.33±0.65	2.86±0.35	2.88±0.35	2.83±0.38
Med. geniculate body	1.82±0.09	5.18±0.37	1.18±0.19	2.57±0.24	2.85±0.61	2.76±0.49
Superior colliculus	1.48±0.11	3.78±0.22	1.85±0.46	2.88±0.37	2.66±0.50	3.00±0.56
Auditory cortex	3.12±0.15	5.79±0.28	0.92±0.17	1.87±0.19	1.14±0.26	1.99±0.53
Inferior colliculus	1.99±0.09	4.76±0.22	2.63±0.44	3.75±0.42	3.77±0.32	4.53±0.11
Visual cortex	1.52±0.08	4.08±0.26	0.53±0.14	1.30±0.22	1.09±0.34	1.07±0.25
Cerebellum	0.82±0.06	2.70±0.33	2.39±0.63	1.96±0.22	3.43±0.41	2.89±0.50

Local CBF is expressed in ml.g⁻¹.min⁻¹. Values are means ± SEM.

prep.). Therefore, no special hypoxic control group was assembled. Since the present study was carried out by the same investigators, using identical techniques, the normocapnic control material was that given in two preceeding communications (Kågström et al. 1982 a and b, submitted). In the following, we will compare the results obtained in the present study to those reported in these communications for recovery lCBF values in normocapnic and normoxic animals.

1. Physiological conditions

Table 1 gives body temperature and mean arterial blood pressure, as well as arterial blood gases and pH, in control and postischemic animals. Differences in body temperature between the

groups were so small that they should be without influence on the results. Hypercapnia did not induce significant changes in blood pressure. However, it should be noted that animals exposed to hypercapnia in the postischemic period had somewhat higher blood pressure than the normocapnic ones reported previously (Kågström et al. 1982 a and b, see Discussion). In the group of animals exposed to complete ischemia, blood pressure was clearly reduced during hypoxia. The degree of hypercapnia was similar in control and postischemic groups.

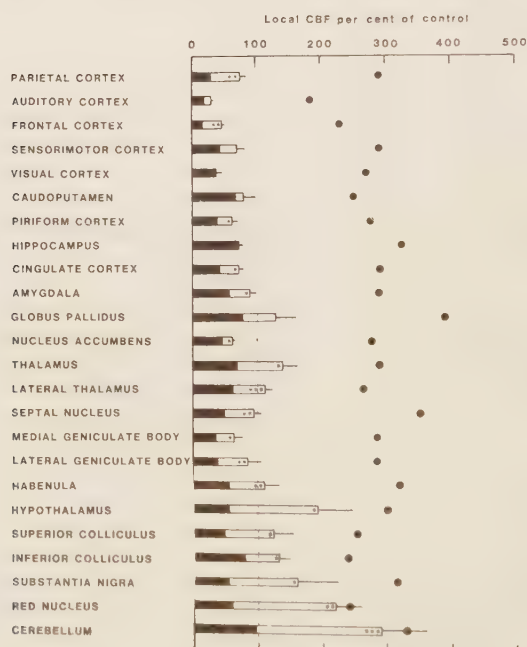


Fig. 1

Local CBF following 15 min of complete ischemia and 60 min of recirculation plus hypercapnia of 2-3 min duration. All values in this graph are expressed in per cent of normocapnic nonischemic controls. The shaded parts of the columns represent mean values for normocapnic postischemic controls (Kågström et al. 1982 a, submitted). Black dots depict mean values for hypercapnic nonischemic controls. Statistical symbols refer to degrees of significance of differences between mean values for hypercapnic and normocapnic postischemic animals, respectively. Values are means $\pm$ SEM.

The severity of hypoxia was one which has been found not to affect cerebral energy state although it leads to moderate lactic acidosis in the brain (MacMillan and Siesjö 1972, Norberg and Siesjö 1975). Hypercapnia, but not hypoxia, was associated with a fall in plasma pH.

2. Local blood flow rates

Table 2 gives data on local CBF in the 24 structures analysed. In all groups but one (incomplete ischemia plus hypercapnia) flow rates within structures were homogenous. When areas with heterogeneous flow rates were examined, the densitometric reading points were chosen to give reasonable approximations to mean flow rates in the structures in question. Such heterogeneities affected only 5 structures, all of cerebral cortical origin and were observed in 5 of 6 animals exposed to incomplete ischemia and hypercapnia. In animals exposed to 15 min compression ischemia erythrocyte volume fraction

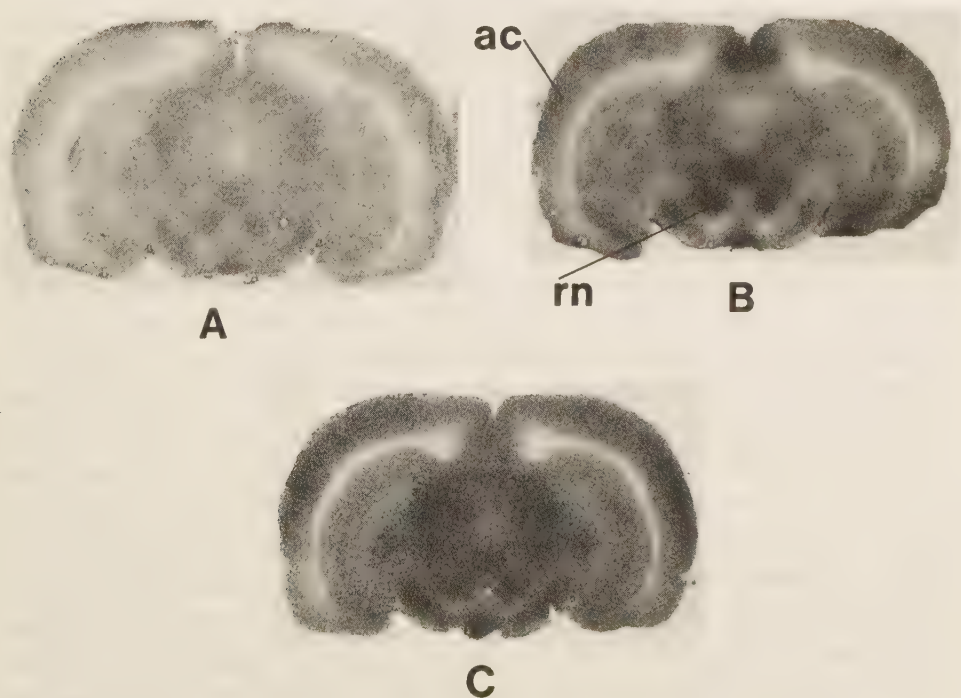


Fig. 2

Autoradiographic pictures of coronal sections from the auditory cortex (ac) and red nucleus (rn) regions after 60 min of recirculation following 15 min of complete ischemia in normocapnic (A), hypercapnic (B), and hypoxic (C) animals.

before, after 15 min, and after 60 min of recirculation was 50 ± 1 , 33 ± 1 , and 38 ± 1 per cent, respectively. In the following, we will discuss under separate headings results obtained in hypercapnia and hypoxia.

a. Hypercapnia

As indicated in the introduction we tested the CO_2 responsiveness of the cerebral vasculature in the recirculation period following complete ischemia to explore whether brain structures, subjected to a uniform degree of ischemia, show differences in their responsiveness. The results of table 2 demonstrate that there were indeed pronounced interstructural variations in CO_2 responsiveness. In order to graphically illustrate these differences we calculated postischemic CBF values in per cent of normocapnic control values, both for normocapnic animals (data from Kågström et al. 1982 a, submitted) and hypercapnic ones (present results). Furthermore, to show how ischemia affected the CO_2 responsiveness we included symbols depicting normal CO_2 responsiveness (hypercapnic control values in per cent of normocapnic controls). The following results emerge (fig. 1). First, in six structures (auditory, sensorimotor, and visual cortex, as well as caudoputamen, hippocampus and globus pallidus) hypercapnia had no significant effect on CBF. It should be noted, though, that in all except two (visual cortex and hippocampus) the mean CBF values were higher in hypercapnic than in normocapnic animals. Second, in 10 structures hypercapnia increased CBF to between 80 and 200% of control, and in most of them the hypercapnic values were about twice as high as those recorded during normocapnia. Third, two structures (nucleus ruber and cerebellum) had normal CO_2 responsiveness. The results thus demonstrate that, even though all brain structures were subjected to complete ischemia of identical duration, the vascular reactivity varied between complete loss and complete preservation of the CO_2 responsiveness. The interstructural inhomogeneity is exemplified in fig. 2, A and B. Hypercapnia elicited a greater increase in CBF in the red nucleus (rn) than in the auditory cortex (ac). This figure (C) also illustrates the homogenous CBF response to hypoxia (see below). (Observe that the densities in the pictures are proportional to flow rates within each autoradiogram, but direct comparisons cannot be made between autoradiograms).

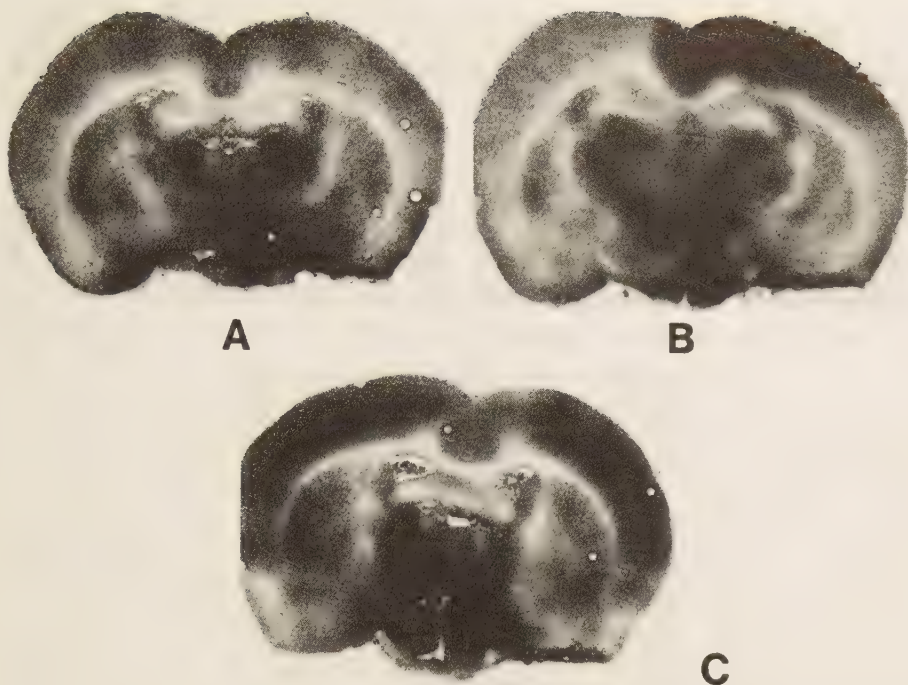


Fig. 3

Autoradiographic pictures of coronal sections from the parietal cortex/thalamus regions following 15 min of incomplete ischemia and 60 min of recirculation plus hypercapnia. The pictures illustrate interanimal and intrastructural inhomogeneities and give examples of marked and uniform attenuation of the CO₂ reactivity in the cerebral cortex (A), heterogenous reaction with zones of low and high flow rates (B), and a homogenous and brisk response to hypercapnia (C).

During incomplete ischemia of the present type the reduction in CBF in different structures varies between virtually complete cessation of flow (e.g. in cerebral cortices) and a very moderate reduction in CBF (e.g. inferior colliculus and cerebellum). (Values for blood flow rates during ischemia in individual structures are depicted in fig. 4, below. Note that in this and other graphs the structures are listed, from top to bottom, in order of flow reduction during incomplete ischemia, see Kågström et al. 1982 b, submitted). The model lends itself, therefore, to studies of the relationships between the severity of the preceeding ischemia and the loss/preservation of CO₂ responsiveness in the period of delayed hypoperfusion. As remarked, 5 of 6 animals showed intrastructural inhomogeneities in flow rates during hypercapnia. Fig. 3, which shows such inhomogeneities, also illustrates the interanimal variation which was observed in this group. One animal showed a marked and fairly uniform attenuation

of CO₂ reactivity in the cerebral cortex (A). A second animal exhibited a heterogenous reaction with zones of high and low flow rates (B) and a third had a homogenous and brisk response to hypercapnia (C).

Fig. 4 shows the general relationships obtained. For those 8 structures in which flow rates during ischemia were consistently below 5% of control (parietal cortex through hippocampus in the figure) CO₂ responsiveness was markedly reduced in the postischemic period (see also nucleus accumbens). It should be noted, though, that in all these

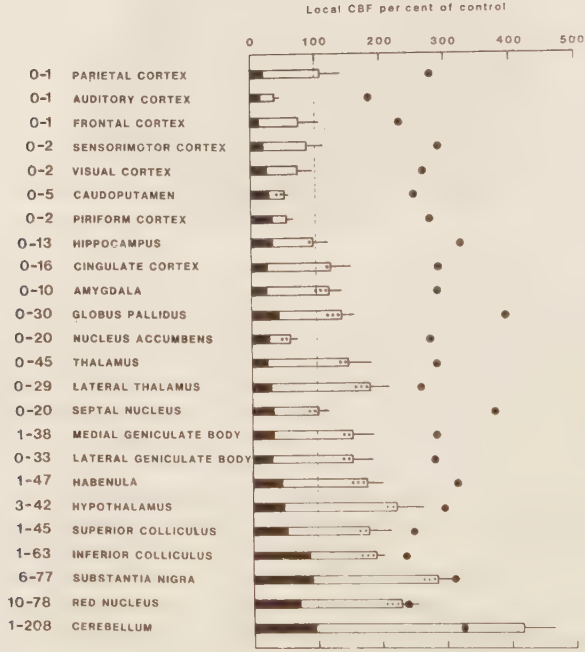


Fig. 4

Local CBF following 15 min of incomplete ischemia and 60 min of recirculation plus hypercapnia. All values in this graph are given in per cent of normocapnic nonischemic controls. The shaded parts of the columns represent mean values for normocapnic postischemic controls (Kågström et al. 1982 b, submitted). Black dots depict mean values for hypercapnic nonischemic control animals. Statistical symbols refer to degrees of significance of differences between mean values of hypercapnic and normocapnic postischemic animals, respectively. Values are means $\pm$ SEM. The figures to the left of the list of structures indicate the range of flow rates in per cent of control during incomplete ischemia in the structure in question.

structures hypercapnia increased mean CBF 1.5 to 3-fold. As mentioned above the response to hypercapnia was heterogenous in 5 structures (parietal, auditory, frontal, sensorimotor, and visual cortices). Since areas with different flow rates were fairly well delimited (cf fig.3 B) it was possible to calculate mean values for zones with high and low flow rates, respectively. The results are displayed in fig. 5 which gives mean flow values in per cent of control in individual experiments, and in structures with heterogenous flow both "high flow" and "low flow" areas are depicted. We note, first, that in all these cortical areas CO_2 reactivity was markedly attenuated, in the whole structure or in a part of it, in at least 4 of 6 experiments. Second, in the hyperemic zones some values approached hypercapnic control values. In these areas, therefore, CO_2 responsiveness was relatively well preserved.

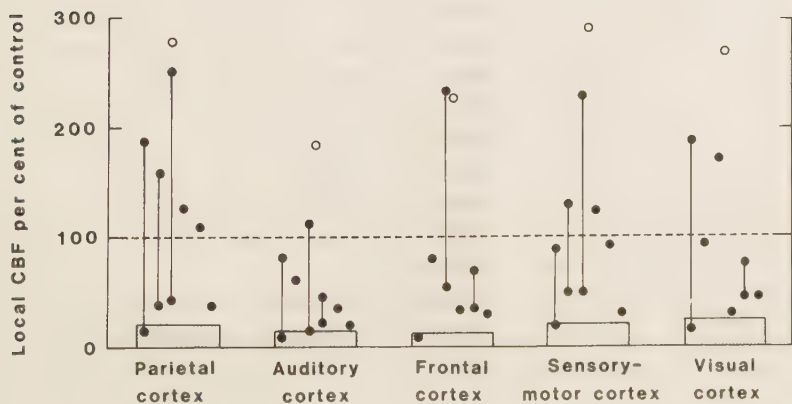


Fig. 5

Local CBF following 15 min of incomplete ischemia and 60 min of recirculation plus hypercapnia in the 5 structures in which intra-structural inhomogeneities was observed. All values are given in per cent of normocapnic nonischemic controls. Filled circles depict mean flow rates in individual animals. In experiments with heterogenous flow values are given for "high flow" as well as "low flow" areas. Unfilled circles represent mean values for hypercapnic nonischemic control animals. Mean values for normocapnic postischemic control groups are given as dashed plateaus.

The results of fig. 4 suggest a rough relationship between the severity of the preceding ischemia and the degree of reduction in CO₂ responsiveness. However, two findings demonstrate that this relationships is not a tight one, or that preservation of CO₂ respon-

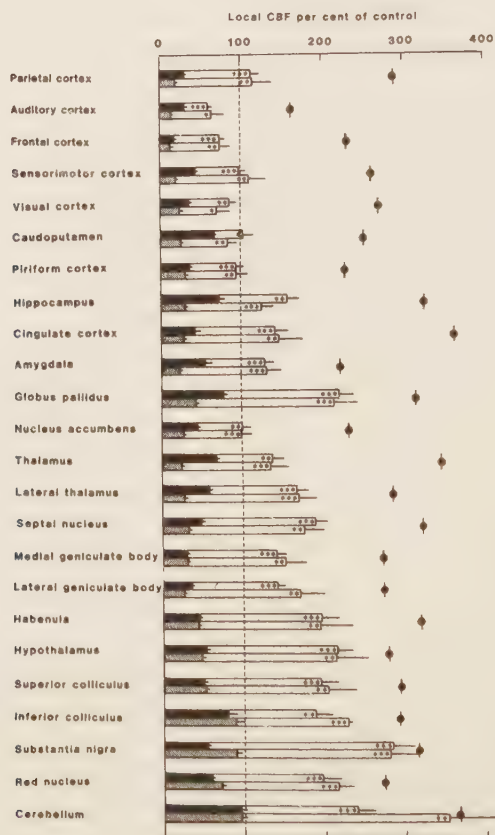


Fig. 6

Local CBF following complete (top column in each pair) and incomplete (bottom column) ischemia and 60 min of recirculation plus hypoxia. All values are given in per cent of normocapnic nonischemic controls. The black and shaded parts of the columns represent mean values for normocapnic animals exposed to complete and incomplete ischemia, respectively, and recirculated for 60 min. Black dots with a crossbar depict mean values for hypercapnic nonischemic controls. Statistical symbols refer to degrees of significance of differences between mean values for hypercapnic and normocapnic postischemic animals. Values are means $\pm$ SEM.

siveness must depend on other factors as well. First, the nucleus accumbens and the septal nucleus showed a strikingly low response. Second, the results obtained in complete ischemia (see above) demonstrate that in some structures preservation of CO₂ responsiveness was not related to the fact that they suffered moderate ischemia only (note particularly red nucleus and cerebellum).

b. Hypoxia

As remarked, flow rates during hypoxia were homogenous within structures. Results obtained following complete and incomplete ischemia were, therefore, displayed in the same graph (fig. 6) which illustrates three main findings, all unpredicted. Firstly, hypoxia increased local CBF to similar values following complete and incomplete ischemia. Secondly, most structures analysed reacted more vigorously to hypoxia than to hypercapnia (fig. 2 C). This is particularly evident when one considers those 8 structures which suffered the most pronounced reduction in CBF during incomplete ischemia. In these, induction of hypoxia increased CBF up to 5-fold. Thirdly, some other structures showed an unexpectedly brisk vascular response to hypoxia, especially if one compares the results to those obtained in hypercapnia. Prime examples of such structures were the globus pallidus and the septal nucleus.

D I S C U S S I O N

As stated in the introduction, three previous studies have shown that complete ischemia attenuates or abolishes the CO₂ responsiveness of the cerebral vasculature (Hossmann et al. 1973, Nemoto et al. 1975, Miller et al. 1980 b). In the first two of these studies global CBF was measured in the recovery period following 3.5-60 min of complete cerebral ischemia and, in the study of Miller et al. (1980 b), cortical CBF was measured at multiple sites in the recovery period following 5 min of ischemia. Without further information, it may be argued from these results that CO₂ responsiveness is partially preserved after 5 min of ischemia but is lost when the period of ischemia is prolonged to 10 min, or more.

The present results make it necessary to revise current views on cerebrovascular reactivity during recirculation, notably in the

period dominated by the phenomenon of delayed hypoperfusion. Thus, our results demonstrate that the CO_2 responsiveness differs considerably between regions, with extreme variations between complete loss and complete preservation of the response. This regional heterogeneity is most readily apparent when one considers the results obtained following complete ischemia, a condition characterized by a uniform reduction in CBF. Another major result emerging from the present study is that the cerebrovascular response to hypoxia is even better preserved. This result is intriguing since it indicates that the vascular responses to hypercapnia and hypoxia are due to mechanisms that are at least in part dissimilar, mechanisms that also seem to be differently affected by the ischemia. We will discuss a few aspects of the results presented.

1. Cerebrovascular response to hypercapnia and hypoxia

Although the brisk responses of the cerebral circulation to hypercapnia and hypoxia have been extensively documented in many animals species, and in man, the mechanisms whereby these conditions lead to vascular dilatation, or those that cause vasoconstriction in hypocapnia and hyperoxia, have remained unclarified. It has been argued by some that changes in extracellular pH constitute a common mechanism, vessel diameter varying directly with the H^+ activity. The evidence in favour of this hypothesis, and results suggesting a role for other mediators such as K^+ , Ca^{2+} , and adenosine, have been extensively reviewed (Lassen 1968, Betz 1972, Kuschinsky and Wahl 1979, Siesjö et al. 1980, Kuschinsky 1981, Winn et al. 1981, Siesjö and Ingvar 1982). These results can be summarized as follows. First, CO_2 seems to act locally, i.e. its effects are not reflexly elicited from extra- or intracellular sites (see Skinhøj and Paulson 1969, Kontos et al. 1977a). Second, since perivascular application of acid solutions dilates vessels it seems likely that the CO_2 effects are mediated by the change in pH (see also results reported by Kontos et al. 1977b). Third, since hypoxia stimulates formation of lactic acid, extracellular acidosis is common to hypercapnia and hypoxia.

Recent results suggest that although gross acidosis undoubtedly dilates cerebral vessels, at least of pial origin, physiological regulation of CBF by extracellular pH may be contributory rather than dominating (see Nilsson et al. 1978, Siesjö and Ingvar

1982). For example, it has been shown that, during hypoxia, CBF may increase precipitously before extracellular pH falls (this is also the case in epileptic seizures). Furthermore, cerebrovascular dilatation also occurs in conditions during which acidosis does not develop (e.g. hypoglycemia). Evidence exists that the mechanisms subserving vasodilatation in hypercapnia and hypoxia are at least partly dissimilar. Thus, whereas the cyclo-oxygenase inhibitor indomethacin dramatically curtails the response to CO₂, it leaves that to hypoxia and to epileptic seizures intact (Sakabe and Siesjö 1979, Ingvar et al. 1980).

Clear evidence exists that the CO₂ responsiveness varies with cerebral metabolic rate. Thus, the response is attenuated by deep anesthesia and in coma (Fujishima et al. 1971, Sandor et al. 1977, Dahlgren and Siesjö 1981). Furthermore, the CO₂ responsiveness of brain structures is directly related to the resting CBF values (Dahlgren et al. 1981), probably because the latter correlates to local metabolic rate (Sokoloff 1981). It has not been established if the vascular response to hypoxia shows a similar correlation to metabolic rate.

2. Loss of CO₂ responsiveness

Several other adverse conditions have been shown to be accompanied by an abolished or attenuated vascular CO₂ responsiveness. These conditions include those following middle cerebral artery occlusion (Fieschi 1971), head trauma (Reivich et al. 1971), and hypoxia (Freeman and Ingvar 1968). Common to these conditions, and those due to ischemia of the present types, is deterioration of cerebral energy state, as well as accumulation of lactic acid and other products of anaerobiosis. It should be noted, though, that loss of CO₂ responsiveness in the postischemic period occurs at a time when the perturbation of cerebral energy metabolism is no longer present (see Ljunggren et al. 1974, and Nordström et al. 1978, for data on present ischemic models). Thus, although the initial reactive hyperemia is probably caused by the results of a perturbed metabolism (increase in extracellular activities of H⁺, K⁺, adenosine, decrease in Ca²⁺ activity) the subsequent reduction in CBF must be viewed as a delayed reaction to events occurring during ischemia, or in the immediate recirculation period.

The question arises whether the delayed postischemic reduction in CBF, and the aborted CO_2 responsiveness, are related to extracellular alkalosis or to depression of metabolic rate. It has been shown that transient ischemia is followed by intracellular alkalosis (Blomqvist et al. 1982, Mabe et al. 1982, submitted); however, extracellular alkalosis has not been documented (Siemkowicz and Hansen 1980, Mabe et al. 1982, submitted). It is also difficult to see how extracellular alkalosis could be compatible with a better preserved response to hypoxia than to hypercapnia. Thus, at least in normal animals, hypoxia of the present degree is accompanied by such moderate lactic acidosis (Norberg and Siesjö 1975) that the change in pH should be much smaller than that accompanying a rise in PCO_2 to 70-80 mm Hg.

It seems equally difficult to argue that the attenuated CBF response to hypercapnia during recirculation can be fully explained by a reduction in cerebral metabolic rate. Thus, this would require extreme variations in postischemic metabolic rate between e.g. the hippocampus (which had an abolished CO_2 responsiveness) and the cerebellum (which had preserved responsiveness) in the recirculation period following complete ischemia. It seems more likely, therefore, that the reduction (or loss) of CO_2 responsiveness is the combined result of postischemic metabolic depression and adverse chemical reactions occurring during ischemia, or in the recirculation period. Obviously, these undefined reactions must differ in severity between brain structures.

3. Interstructural differences in vascular responsiveness

A major result of this study concerns the pronounced interstructural variability in vascular response to both hypercapnia and hypoxia, another the differences in responses in the two conditions. The results demonstrate that the interstructural differences, notably those observed after complete ischemia, can neither be related to the severity of the delayed hypoperfusion (see fig. 1 and 4) nor to the preischemic local metabolic rate (Sokoloff 1981; for data on ventilated rats under 70% N_2O , see Ingvar et al. 1980).

As indicated in the introduction, studies of local vascular reactions in postischemic conditions may give clues to the undefined phenomenon of selective neuronal vulnerability. Unfortunately, histopathological studies have not been carried out that define the final cell damage incurred by the present types of ischemia. However, such

results are available for an ischemic model similar to the present model of incomplete ischemia. In that study (Pulsinelli et al. 1982 b), neuronal cell damage was largely confined to the hippocampus, to the neocortex and the caudoputamen. We note that in these structures, CO₂ responsiveness was either lost or grossly attenuated. Although this relationship is suggestive, further conclusions will require histopathologic data on recovery following complete ischemia.

In conclusion, our results require revision of current concepts of vascular "unreactivity" in the postischemic recovery phase since they demonstrate a pronounced interstructural variability in CO₂ responsiveness, and a better preserved response to moderate hypoxia. In view of these results, it seems justified to redirect studies on the relationships between metabolic, circulatory, and structural sequelae of cerebral ischemia to the regional or local level.

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